Assessment of Maize Germplasm Lines for Genetic Diversity, Cultivar Superiority and Combining Ability

By

Suzan Khoza

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School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

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GENERAL ABSTRACT

Maize (*Zea mays* L.) is an important crop in the world; however, its yield is compromised by new production challenges leading to poor yield in sub-Saharan Africa. This calls for a need to enhance maize adaptation to changing climate and challenging environments. The new maize varieties should be richly endowed with high frequency of genes that confer high yield under stress and non-stress conditions. Currently, such maize is not available, prompting research into development of new germplasm lines for use in developing new hybrids. The objective of the study was to determine i) the level of genetic diversity using SSR molecular markers and phenotypic data in a set of 60 maize inbreds from the breeding program, ii) genotype by environment interaction in maize hybrids, iii) cultivar superiority, iv) combining ability effects, v) the relationship between yield and secondary traits and vi) the relevant genetic parameters that underpin genetic gains in a breeding program. To study genetic diversity present in the germplasm, phenotypic data and 30 SSR markers were used to estimate the genetic distance between the inbreds. The results indicated that inbred lines which were put in the same cluster were related by pedigree and origin. To assess the level of genotype by environment interaction (GXE) and cultivar superiority of the new germplasm lines, hybrids were planted in five environments with two replications. Data were analysed using the REML and AMMI tools in GenStat 14th edition. The results revealed significant differences between hybrids and environments for grain yield. However, GXE interaction was also significant indicating possible challenges which can be encountered in selecting new hybrids. To determine combining ability estimates two different testers were used. The REML tool from GENSTAT was used to perform the line X tester analysis. Results indicated that both additive and non-additive gene action were important for grain yield. The direct selection strategy for yield was recommended because heritability of grain yield was high. Overall, results suggested that the information on genetic diversity will assist in defining heterotic groups; which will enable effective and efficient management of the germplasm lines to produce new maize hybrids.

DECLARATION

I, Suzan Khoza, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted. Then:
   a. Their words have been re-written but the general information attributed to them has been referenced;
   b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

4. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the references sections.

Signed

……

Suzan Khoza

As the candidate's supervisors, we agree to submission of this dissertation:

…….

Prof. John Derera (Supervisor)

……..

Prof Mark Laing (Co-Supervisor)

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DEDICATION

Most importantly I would like to dedicate this work to our Heavenly Father who made everything possible, and carried me through all the challenges and my supportive and loving father Julius Khoza and mother Florah Khoza.

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LIST OF ABBREVIATIONS

AD: Anthesis date

ANOVA: Analysis of variance

AMMI: Additive Main effects and Multiplicative Interaction model

ASI: Antheis-Silking Interval

CV: Coefficient of variation

CIMMYT: International Maize and Wheat Improvement Center

DNA: Deoxyribonucleic acid

EH: Ear height

EPO: Ear position

EPP: Number of ears per plant

ER: Ear rots

FAO: Food and Agriculture Organization of the United Nations

FAOSTAT: Food and Agriculture Organization Statistics

GCP: Generation Challenge Program

GCA: General combining ability

GD: Genetic distance

G X E: Genotype by environment interaction

GY: Grain yield

H^2: Heritability in a broad sense

IPCA: Interaction principal component axis

LSD: Least significant difference

L X T: Line by tester

MAS: Molecular marker-assisted selection

MOI: Grain moisture

PCR: Polymerase chain reaction

PH: Plant height

RFLP: Restriction fragment length polymorphism

RAPD: Randomly amplified polymorphic DNA

REML: Restricted Maximum Likelihood

RL: Root lodging

SCA: Specific combining ability

SL: Stem lodging

SSA: Sub-Saharan Africa

SSR: Simple sequence repeats

TEX: Grain texture

INTRODUCTION TO DISSERTATION

1. Importance of Maize

Maize originated in Mexico and it is widely grown from 58°N and 40°S, under low and high altitude. Maize, together with rice and wheat, provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al., 2011). It plays an important role in the livelihoods of many poor farmers. This is because about 67% of the total maize production in the developing world comes from low and lower middle income countries (Shiferaw et al., 2011). Statistics indicates that of the 23 countries with a high *per capita* consumption of white maize, 16 are in sub-Saharan Africa (Sibiya, 2009). There are many ways in which maize can be utilized in comparison to other cereals. Virtually all plant parts of maize have economic value (M'mboyi et al., 2010). In sub-Saharan Africa, maize is used mainly for human consumption; while in industrialized countries it is used as livestock feeds and as raw material for industrial products (M'mboyi et al., 2010). Maize is an important source of carbohydrate, protein, iron, vitamin B, and minerals (M'mboyi et al., 2010). Consequently, the demand for maize continues to increase in the world.

The maize grain yields are extremely low, averaging approximately 1.5 tons per hectare in Africa, yet maize is the backbone of basis for food security in some of the world's poorest regions in Africa, Asia and Latin America (Shiferaw et al., 2011). The United States of America remains the highest maize producing country in the world, with more than two times world's grain yield/ha (Figure 1). South Africa, Mexico and the rest of Africa have considerably lower yields of maize relative to the United States. However, South Africa realizes the highest grain yield/ha in Africa. The average grain yield/ha of the rest of the world is approximately two times that of Africa (Figure 1).

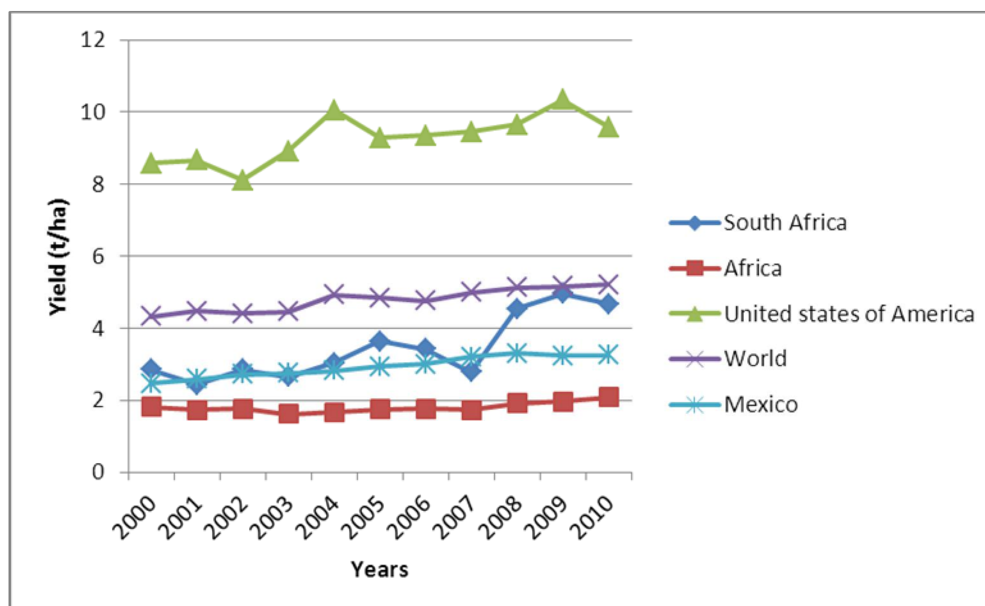

Figure 1: The grain yield/ha of maize around the world (FAOSTAT, 2012)

Low grain yields in Africa are attributable to many factors including biotic and abiotic factors (M'mboyi et al., 2010). Abiotic factors include drought, extreme temperatures, low soil fertility, soil acidity, and flooding (M'mboyi et al., 2010). Yield losses due to abiotic factors are normally confounded with those from biotic factors, such as high incidence of diseases, insect pests and weeds, which results in yield reduction by more than 30% (Shiferaw et al., 2011). Therefore, new strategies or improvement in the existing strategies is required to deal with these challenges that threaten food security.

2. Rationale of the study

Climate change presents challenges in food security in Sub-Saharan Africa (SSA) as reflected by global warming; increasing frequency of drought in maize production areas in South Africa, consequently there is a need to develop new maize cultivars that can cope with climatic change and other environmental challenges. The new maize varieties should be endowed with high frequency of genes (or alleles) that confer high yields under stressful conditions of heat, drought and biotic stresses. Such maize cultivars are not currently available in South Africa; hence a new breeding program was established at the University of KwaZulu-Natal in 2007 to introgress temperate germplasm into the subtropical populations

to broaden the diversity of the maize germplasm for improved yields. The main breeding objective is to generate new sources of germplasm for use in breeding new resilient varieties that are adapted to South African ecosystems amidst the challenges that are presented by stress. To achieve the above objectives, new maize inbreds were derived using conventional breeding methods from many populations. The inbreds were derived directly from genetically broad-based populations or from bi-parental pedigree crosses among the elite hybrids. Temperate germplasm materials were introgressed and incorporated into the tropical base germplasm collection to introduce the new alleles for early maturity to fit the germplasm into increasingly short seasons in SSA (Lewis and Goodman, 2003) and to facilitate late season planting. The other broad objective was to enhance standing ability and prolificacy to ensure adaptation under windy and low planting population conditions respectively, in South Africa. Overall there is need to expand the plant variety technology options for the farmers in South Africa because of their capacity to supply food to the rest of the continent.

Introgression of new genetic resources enables widening of the available genetic base and facilitates the identification of materials that are adaptable to drought and heat, effects of climate change such as global warming and increasing frequency of drought in African countries. Shin et al. (2006) reported that it is very important to measure genetic diversity in populations of a crop species in order to understand its genetic structure and subsequently improve it by genetic manipulation. In addition, there is an important role of understanding the genetic diversity among and within inbred lines at the molecular level for maize improvement in different breeding programs (Shehata et al., 2009). This is so that inbred lines from different heterotic groups can be combined and form distinct heterotic patterns. Due to the large genotype x environment interaction, diversity studies that are based on phenological and morphological characters usually varies with environments, and evaluation of these traits require growing the plants to full maturity prior to identification. As a result, breeders are interested in new technologies that can make this process more effective (Ibitoye and Akin-Idow, 2010). For this reason molecular-marker tools in combination with phenotypic traits are frequently being used to determine the diversity and genetic distances among the germplasm lines and populations. In the last two decades molecular markers

have proved to be very useful for genome characterization and breeding (Araus et al., 2008), hence, they have been effectively integrated with classical tools in the current study.

3. Research objectives

The main objective of this research is to determine the diversity, cultivar superiority and combining ability in the new maize inbred lines derived from diverse crosses among subtropical and temperate populations at the University of KwaZulu-Natal (UKZN) breeding program. This information is crucial in devising a new breeding strategy that will enhance the effectiveness of the programme.

The following specific objectives were pursued:

a) To investigate genetic diversity using SSR molecular markers and phenotypic traits in a set of 60 maize inbreds from the program at UKZN
b) To determine genotype by environment interaction and cultivar superiority in maize hybrids
c) To determine combining ability of maize germplasm lines
d) To determine the relationship between yield and secondary traits in maize hybrids
e) To determine genetic parameters such as heritability, genetic and phenotypic coefficient of variation that underpin genetic gain in a breeding program.

4. Research hypotheses

The research hypotheses were as follows:

a) There is genetic diversity in a set of 60 maize inbreds from the program at UKZN.
b) There is genotype by environment interaction in maize hybrids which would impact on their yield stability in deferring production environments
c) There are superior new hybrids which exhibit high grain yield when tested in different environments
d) There is high combining ability within the new germplasm lines which is crucial for developing hybrids

e) There is a strong relationship between yield and secondary traits in hybrids which can be used to conduct indirect section
f) There are significant genetic parameters that underpin genetic gain in a breeding program which can be crucial in devising suitable breeding strategy

5. Structure of the dissertation

This dissertation is made up of literature review, three research chapters and overview of the study as follows:

a) Assessment of genetic diversity in maize population using Molecular markers and phenotypic traits
b) Assessment of Maize Testcrosses for GXE and Cultivar Superiority
c) Combining Ability and Genetic Variation among New Germplasm Lines
d) General overview of the study and future directions

All, except for chapter one (literature review), are written in IMRD format that include Introduction, Materials and Methods, Results and Discussion. All chapters have a reference list and contain some limited repetition and overlap of some content which is unavoidable.

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CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

The literature related to the objectives of the present study is reviewed, under the following headings: Genetic diversity in maize, Genetic Distance in maize, Heterosis, Heterotic grouping and patterns, Relationship between Genetic Distance and heterosis, Combining Ability, Gene Action, Diallel and Line X Tester Analysis, Relationship between yield and secondary traits in maize, Genotype X Environment Interaction and Cultivar superiority and rank analysis. Conclusions drawn from this review are provided at the end of the chapter.

1.2 Genetic diversity in maize

The information on genetic diversity is very important for germplasm enhancement (Hoxha et al., 2004; Shin et al., 2006; Dagne, 2008; Kumar et al. 2009; Makumbi et al., 2011). Frankham et al. (2002) defined genetic diversity as “the variety of alleles and genotypes present in a population, reflected in morphological, physiological and behavioral differences between individuals and populations”. Assessing the levels and patterns of genetic diversity accurately is important for managing a maize breeding programme. Prasanna et al. (2002) cites the following three reasons for studying diversity: (i) maintenance and broadening of the genetic base of the elite germplasm; (ii) selection of appropriate parental lines for hybrid combinations; and (iii) generation of segregating progenies with maximum genetic variability for further selection. Progress in breeding is realised if there is sufficient genetic variation and diversity (Cholastova et al., 2011). This is because the selection of improved genotypes depends on the availability of genetic variability (Cholastova et al., 2011). Genetic diversity has been compromised in maize due to intensive breeding for similar environments worldwide. For example, Li et al. (2002) reported that in China, the parenthood of more than 90% of the hybrids consists of about 20 elite inbred lines. Similarly, the pedigrees of most hybrids in the United States are derivatives of 6–8 inbred lines (James et al., 2002; Rasmussen and Hallauer, 2006) which demonstrate the observation of narrow genetic bases in maize. Almeida et al. (2011) used SSR markers and reported low genetic diversity 0.22 to

0.33 in normal maize and sweet corn compared to the main populations of CIMMYT (International Maize and Wheat Improvement Center), which displayed genetic distances of 0.45 to 0.61 with SSR markers. Limited genetic diversity would negatively impact on maize breeding in the future.

Maize is out crossing in nature and heterozygous, hence possesses broad genetic diversity (Jebaraj et al., 2010; Cholastova et al., 2011), but this is reduced by selection. Additionally, Parvez et al. (2006) explained that it possesses enormous genetic variability which breeders would exploit. However diversity is compromised by emphasizing on maximum productivity, quality and uniformity requirements (Cholastova et al., 2011) during selection. Maize molecular diversity is roughly 2 to 5 fold higher than that of other domesticated grasses (Figure 1.1); however, these folds are reduced by modern breeding which focuses on a few traits. Maize's closest wild relative, *Z. mays* ssp. *parviglumis*a (teosinte), often has levels of nucleotide diversity that surpasses 2% (3–6) (Flint-Garcia et al., 2009) because less selection has been made on it. The great diversity of maize and teosinte provided raw genetic material for the radical transformation of maize into the highest yielding grain crop in the world (Flint-Garcia et al., 2009). Therefore, this suggests that modern maize can also be backcrossed to teosinte to expand its genetic variation and get genes it lost through continuous selection.

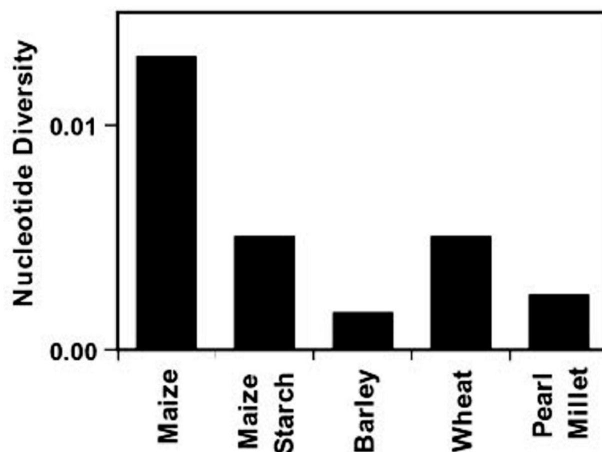

Figure 1.1: Comparison of nucleotide diversity in maize and various grass crops (Flint-Garcia et al., 2009).

1.2.1 Estimation of genetic diversity

Genetic diversity between taxonomic units before 1970 was measured using methods which relied on pedigree analysis, and morphological, physiological or cytological markers as well as biometric analysis of quantitative and qualitative traits, heterosis or segregation variance in crosses (Melchinger, 1999 as cited by Legesse et al., 2008). However, there are limitations to using pedigree information for genetic distance. The calculations of pedigree relatedness may not be valid (Legesse et al., 2008). Systemic relationship in maize has long been estimated using morphological traits but their discrimination capacity is limited (Cholastova et al., 2011) by the presence of genotype x environment interaction. Consequently, biochemical and cytological markers are used to monitor germplasm biodiversity (Cholastova et al., 2011). The two types of markers cannot be used to study the complete genome of a species due to their limitation in number. The isozymes have low polymorphism which prompted the development of molecular markers such as simple sequence repeats (SSR), Single nucleotide polymorphism (SNP) among others (Melchinger, 1999 as cited by Legesse et al., 2008). These are more effective because they are infinite and have better genome courage and can be used in defining heterotic groups and examining relationships among inbreds at the DNA level. Smith et al. (1997) reported that SSR markers were effective for discriminating US and European maize germplasm, for this reason SSR markers were adopted for use in the current study.

1.3 Genetic distance

Grouping of similar germplasm is a first step in identifying promising heterotic patterns as determined by genetic distance (GD) based on molecular markers (Legesse et al., 2008). This can be very effective as only genotypes with known GD can be crossed to make hybrids with high possibility of success. Genetic distances between genotypes have been widely used for reconstructing breeding histories, describing patterns of genetic diversity, and assigning lines to heterotic or other biologically or economically important groups (Cheres et al., 2000). The GD can be estimated without phenotyping the germplasm to be classified (Cheres et al., 2000) as this method can be time consuming and less accurate. Nonetheless, the main multivariate technique used to measure GD is based on phenotypic characters (Bertan et al., 2007). Genetic distance studies for choosing parents involve the following six steps: i) “election of genotypes to be analyzed; ii) data production and formatting; iii)

selection of the distance definition or measurement to be used for the estimations; iv) selection of the clustering or plotting procedure to be used; v) analysis of the degree of distortion caused by the clustering/plotting procedure used and vi) interpreting the data" (Cruz and Carneiro, 2003 as cited by Bertan et al., 2007). Multivariate analysis is the major tool used in estimating GDs as it allows for the possibility of gathering many variables into one analysis (Bertan et al., 2007). Genetic distance can be estimated from various types of molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) (Semagn et al., 2012). However, in the current study SSRs were used to determine GD which is complemented by phenotyping.

1.3.1 Estimation of genetic distances

The most statistics used to estimate GD are Mahalanobis (D^2) and the Euclidean distance (Darbeshwar, 2000; Bertan et al., 2007). However, the Mahalanobis distance has some advantage over the Euclidean distance as it takes into account the environmental effects and allows for obtaining correlations between characters (Bertan et al., 2007). Unfortunately, Mahalanobis procedure requires data of more than one replication to estimate the distance (Bertan et al., 2007), hence its use is limited. The data is then presented in a symmetrical matrix once the distance estimate between each genotype pair is obtained and then analysed by the use of a clustering/plotting procedure (Bertan et al., 2007). Pool observation can be separated into many subgroups to obtain homogeneity within and between the formed subgroups using the clustering method (Darbeshwar, 2000; Bertan et al., 2007). Breeders use hierarchical methods to group genotypes by a process that repeats itself at many levels, forming a dendrogram without concern for the number of groups formed (Bertan et al., 2007). Different clustering methods can be used depending on the procedure that is most suitable to the data set i.e. Tocher's clustering leads to the formation of one large cluster, whereas the UPGMA better discriminates the closer genotypes (Bertan et al., 2007). Hence, UPGMA was adopted in this study and some of the materials were closely related.

1.4 Heterosis

Heterosis was coined by Shull in 1914 and is described as the superiority of the F_1 hybrid performance over its parents (Zhang et al., 2002). The inbred-hybrid concept was proposed along with the rediscovery of Mendelian law of inheritance (Zhang et al., 2002). The level of heterosis depends on the parents chosen and trait measured (Farhan et al., 2012) which is why it is very important to select good parents. The successful exploitation of heterosis has enhanced yield in maize (Premlatha et al., 2011) because inbred lines from different heterotic groups have a high potential of producing hybrids with greater vigour.

Heterosis is determined by the average performance of the two parents or high parent heterosis (Fato, 2010), hence the levels of differences between the parents is important. According to Hallauer and Miranda (1988), genetic divergence of parental varieties determines the manifestation of heterosis and the heterotic patterns determine the genetic divergence of two parental varieties. In other words the larger the heterotic patterns between two parental varieties the more genetically diverse they are. The maize breeding germplasm base can be broadened by the knowledge of heterotic patterns (Mungoma and Pollak, 1988) because if poor heterotic patterns are observed in the program new germplasm can be introgressed. In breeding programs heterosis can be exploited by generating lines from different heterotic groups and crossing them to produce a high yielding hybrid (Fato, 2010) and heterotic groups can be identified through the knowledge of genetic distances between the inbred lines.

Heterosis for grain yield and other quantitative traits has been comprehensively studied in maize (Reif et al., 2005; Jebaraj et al., 2010). Although many hypotheses have been suggested to explain heterosis, its genetic, physiological, and biochemical bases still remain largely unexplained (Reif et al., 2005) and the molecular genetic base of heterosis is still not understood (Drinic et al., 2002). As a consequence, heterosis has not been exploited effectively (Fato, 2010). This implies that understanding heterosis can further increase yield in maize as it will be exploited fully.

1.5 Heterotic grouping and patterns

A heterotic group was defined as "a group of related or unrelated genotypes from the same or different populations which show similar combining ability or heterotic response when crossed with genotypes from other genetically distinct germplasm groups" (Melchinger and Gumber, 1998). Heterotic patterns refer to specific pair of inbred lines or varieties which expresses high heterosis in crosses. Genetically distinct lines have a high potential of expressing high heterosis, whereas genetically similar lines expresses inbreeding depression. Inbreds which were selected from a divergent background have different heterotic groups and are therefore preferred (Fato, 2010). This means that different heterotic groups can be formed based on the gene frequency of parental genetic materials employed to make crosses (Fato, 2010). This can effectively be done by studying genetic distance between the lines before crosses are made. When inbred lines are classified into heterotic groups, this facilitates the exploitation of heterosis in maize and can contribute to hybrid performance (Bidhendi et al., 2011). Reif et al. (2005) cited the advantages of grouping germplasm into divergent heterotic groups as follows: (i) "a higher mean heterosis and hybrid performance and (ii) a reduced specific combining ability (SCA) variance and a lower ratio of SCA to general combining ability (GCA) variance". Moreover, this is because if lines are grouped in different clusters, evaluation and establishment of unnecessary hybrids can be avoided (Aguiar et al., 2008). An advantage of using molecular markers over conventional methods is that few divergent lines are discriminated, and this leads to the formation of heterotic groups that contain genotypes, which explicitly represent the differences in the allele frequency of the populations (Aguiar et al., 2008). However, in the current study both molecular and conventional methods have been used to study genetic diversity.

1.5.1 Methods Used in Heterotic Grouping

The efficiency in producing hybrids, new inbreds and population of hybrid development can be increased by exploiting heterotic pattern (Zhang et al., 2002), because this help identify inbred lines that have a good combining ability. In several studies heterotic groups were

identified based on pedigree and origin. Wu (1983) classified inbred lines into 4 or 5 groups based on pedigree data and to predict heterotic patterns used in China. This is because inbred lines from the same origin are likely to belong to the same heterotic group as they have the same adaptation.

Zhang et al. (2002) reported that Cluster analysis based on SCA can be used to classify inbred lines into heterotic groups. They added that diallel analysis is available for use; however, the reliability for SCA analysis depends upon the quantity of inbred lines used as parents and the genetic base of the entries. As a result the application of diallel analysis is restricted. However, Betrán et al. (2003) reported that SCA approach is more reliable than heterosis which is influenced by the environment. Thus, use of SCA based methods is more recommended because SCA effects have better predictive value for F1 grain yield than heterosis (Betrán et al., 2003). The design II (North Carolina mating) is another approach that can be used, but the precondition for this approach is the availability of a set of reliable common testers (Zhang et al., 2002), hence it may not be useful for the identification of new heterotic patterns.

Molecular marker technology provides a kind of genetic markers based on DNA structure polymorphism. Molecular markers are not influenced by change in season and environment and can be detected at any stage of plant development (Zhang et al., 2002) hence are more reliable. Inbred lines can be assigned to heterotic groups based on the genetic divergence among lines, based on the data obtained by molecular markers (Pinto et al., 2003). The use of molecular tools to determine heterotic groups is based on genetic distances rather than heterosis. However, the results from molecular tools have not been consistent in associating grain yield with genetic distances (Fato, 2010). This therefore means that molecular markers may not be useful in predicting heterosis among inbred maize line or identifying effective heterotic groups. It was found that heterosis could be obtained even between parent-inbred lines from the same group from the analysis of the testcross (Fato, 2010). Specific combining ability estimates require the production and evaluation of crosses in the field trials, which makes the process costly. The advantage of using molecular markers is the possibility of evaluating only the more promising crosses between the most divergent lines (Pinto et al.,

2003) and hence cost and time effective. The fore going emphasizes that no one method can be used to estimate heterotic grouping however integration of different methods can be the best solution because each method has its limitations.

Barata and Carena (2006) reported that groups of genetically similar germplasm using SSR markers could not be identified accurately and reliably even when the available germplasm was diverse. They concluded that extensive field evaluation is recommended to classify unrelated inbred lines of maize. In contrast, Reif et al. (2003) concluded that there is an excellent agreement between relationships obtained by SSR data and pedigree information. Similarly, Aguiar et al. (2008) did a study to determine heterotic groups of germplasm lines of tropical maize by test crosses and by simple sequence repeat (SSR) markers and to compare five grouping methods of heterogeneous maize. They found that grouping by SSR markers was consistent with the genealogy of the lines and is a useful procedure for the formation of heterotic groups of tropical maize lines (Aguiar et al., 2008). Therefore SSR markers can be complemented with field trials to identify heterotic groups and to introgress exotic germplasm (Reif et al., 2003). For this reason SSR markers complemented with field trials were adopted for the current study.

1.6 Relationship between Genetic Distance and Heterosis

Genetic distance (GD) has been extensively correlated with heterosis in several crops such as maize, oat, rice and wheat based on molecular markers, however, the results varied (George et al., 2011). The general conclusion based on RFLP and SSR marker data from previous studies was that heterosis was significantly related to the heterozygosity of marker loci, but the relationship is a complex one (George et al., 2011), implying that heterosis is largely expressed between two divergent lines. The estimates of correlation between GD and heterosis were statistically significant but weak in many cases (George et al., 2011). It is a challenge for maize breeders to predict and identify inbred lines that can produce highly heterotic hybrids (Xu et al., 2004). This is because it cannot be guaranteed that inbred lines from different heterotic group will always produce high yield.

Lamkey and Lee (1993) reported that molecular marker-based GD has some potential for predicting hybrid performance of related lines, however, has no value in predicting hybrid performance produced from unrelated lines from different heterotic groups. This is in agreement with Benchimol et al. (2000) who investigated genetic distances among tropical maize material and their relationship to heterotic group allocation and hybrid performance using RFLP molecular markers. The results showed that there are correlations of parental GDs with single crosses and their heterosis for grain yield, which were high for line crosses from the same heterotic group and low for line combinations from different heterotic groups. This shows that RFLP-based GDs are efficient and reliable to assess and allocate genotypes from tropical maize populations into heterotic groups but are not suitable for predicting the performance of line crosses from genetically different heterotic groups (Benchimol et al., 2000).

Furthermore, Xu et al. (2004) did a study to survey the genetic diversity among 15 elite inbred lines of maize in China with SSR markers and assessed the relationship between SSR marker and hybrid yield and heterosis in a diallel set of 105 crosses. They found that the cluster diagram based upon the SSR data grouped the 15 lines into families consistent with the yield heterotic response of these lines. In addition, GD based on SSR data was significantly correlated with hybrid yield. Similarly, Shehata et al. (2009) reported that the application of six different SSR markers successfully provided the information on similarity or diversity as well as the heterozygosity of the allelic loci for all the eight maize inbred lines. However, the correlation between GD and heterosis is affected by several factors. For example, George et al. (2011) studied the effect of phosphorus stress on the relationship between GD and hybrid performance. They found that the utility of GD as a predictor of hybrid value is best up to a certain threshold, as correlations with GD became inconsistent when the inbred parents were greatly divergent. There was no correlation between GD and F1 grain yield, mid parent heterosis, high parent heterosis and SCA when the GD was >0.77. The high correlation of GD with F1 grain yield and with SCA in specific subsets of crosses having a narrower range of GD shows that GD can be put to practical use in predicting hybrid performance.

Nonetheless, another survey of the literature indicate that there is a high correlation between genetic distance and hybrid performance in maize (Xu et al., 2004) but there are reports that support limited utility of GD in hybrid development. Genetic distance measures have been reported to be of limited use in predicting hybrid performance, heterosis and SCA of single crosses (Legesse et al., 2008). To explain the inconsistence relationship between genetic distance and heterosis Betrán et al. (2003) reported that the degree of heterosis depends on the relative performance of inbred parents. The corresponding hybrids environments can differentially affect the performance of inbred lines and hybrids, altering the relationship between GD and heterosis (Betrán et al., 2003). They concluded that the influence of abiotic stresses on the use of GD as a predictor of hybrid performance is not well understood. Furthermore, Darbeshwar (2000) made a conclusion that there is an optimum degree of genetic divergence for a maximum expression of heterosis and this optimum occurs within a range that is narrow enough so that the incompatibility barriers are not apparent. Within this range the amount of heterosis is linear function of the difference in allele frequency. Given the foregoing it is not conclusive whether GD would be useful in identifying good hybrids, perhaps due to complications resulting from GXE.

1.7 Combining ability

Grain yield of maize has been increased by hybrids over the years (Kanagarasu et al., 2010). The combining ability of an inbred is measured as its ability to combine with other inbreds and produce superior hybrids (Bello and Olaoye, 2009). Additionally, combining ability is the relative ability of a genotype to transmit its desirable performance to its crosses. Sprague and Tatum (1942) defined general combining ability (GCA) as “the average performance of a genotype in hybrid combination while specific combining ability (SCA) as those cases in which certain combinations perform relatively better or worse than would be expected on the basis of the average performance”. Maize breeders are interested in identifying inbred lines that would combine well and give high yields without making all possible crosses among the potential parents (Makumbi et al., 2011). This is because breeders are always looking for effective methods which are reliable and cost effective. Combining ability analysis is the quickest method of understanding the genetic nature of quantitatively inherited traits and also gives essential information about the selection of parents which can

give better segregants in a hybrid combination (Kanagarasu et al., 2010). It also allows for grouping of inbreds with similar combining ability.

In a hybrid oriented program where hybridization and selection are emphasised, combining ability estimation is important (Farhan et al., 2012) as it gives an indication of lines which combine well for different traits. Knowledge about the combining ability of parents, their behaviour and performance in hybrid combination is important for designing new hybrids (Bello and Olaoye, 2009; Legesse et al., 2009; Jebaraj et al., 2010; Khalil et al., 2010). This knowledge is used to select suitable parents for hybridization and in selecting promising hybrids for advancement in the programme (Bocanski et al., 2011). Selection of suitable parents based on combining ability data helps to know the genetic architecture of various characters that enables the breeder to design effective breeding plan (Amiruzzaman et al., 2011). Bidhendi et al. (2011) also reported that combining ability of new and elite lines should be established to enhance strategic planning of a breeding program.

1.8 Gene action

The genetic structure of the crosses analyzed and the environmental conditions in which they were grown determines the proportion of additive and non-additive components of genetic variance (Khotyleva and Trutina, 1973). General combining ability is mainly influenced by additive gene effects and additive X additive interaction variance; while SCA is influenced by dominance variance, and epistasis components such as additive X additive variance, additive X dominance variance and dominance X dominance variance components (Rojas and Sprague, 1952; Darbeshwar, 2000). The variance due to GCA is usually considered to be an indicator of the extent of additive type of gene action, whereas SCA is taken as the measure of non-additive type of gene action in hybrids breeding (Kanagarasu et al., 2010). This means that inbred lines with good GCA have superior genes coming either from lines or testers used to produce good hybrids. On the other hand lines with good SCA are as a result of the interaction between the line and the tester. Predominance of additive gene action is more important in programmes that emphasise selection to develop populations; whereas non-additive gene action is crucial for hybridisation strategy in inbred line crosses (Kebede, 1989). Younes and Andrew (1978) reported that for most traits in

previously unselected material, additive gene action is more important than non-additive components. Furthermore, Amaregouda (2007) explained that if both parents of the heterotic hybrids involve high GCA effects, then it implies that the parental contribution to heterosis is mainly through additive gene action. Variance components due to GCA for grain yield were found to be larger than those due to SCA (Aly et al., 2011) implying that additive gene action were predominant over the non-additive, respectively. It is more meaningful to consider heterosis and combining ability together (Amaregouda, 2007) because the information about combining ability is used to judge whether hybridisation would be successful or not. Abdel-Moneam et al. (2009) found that GCA and SCA mean squares were highly significant for ear length, grain yield and shelling percentage, but the SCA was more important indicating that these traits were predominantly controlled by non-additive gene action.

1.9 Diallel and Line X Tester Analysis

Diallel mating design, which entails all possible crosses among a set of inbred parents, has been used to estimate combining ability in maize and other crops. It provides information on the performance of parental populations and their heterotic pattern in crosses. It also identifies heterotic groups and predicts performance of new populations (Bello and Olaoye, 2009). The disadvantages of diallel analysis involves the workload during evaluation and the fact that some hybrids may be difficult to obtain (Bertan et al., 2007), hence other methods may be used to compensate for the weakness of diallel analysis.

Line × tester mating design was developed by Kempthorne in 1957 and it provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations (Farhan et al., 2012). Packer (2007) defined Line x Tester analysis as the interaction between the experimental lines and the testers for the dependant variable in a statistical model. A significant line x tester interaction provides evidence that the ranking of experimental lines differs depending on the tester used (Packer, 2007), hence an appropriate tester must be selected to evaluate new germplasm lines (Aly et al., 2011). The testers that can be used in a breeding program may they can either be genetically narrow or broad-based, related or unrelated to the lines being evaluated, have a high or low frequency

of favourable alleles and high or low yielding (Packer, 2007; Aly et al., 2011). In general the combining ability between the line and the tester will determine the performance of the hybrid that will result.

Rawlings and Thompson (1962) as cited by Packer (2007), pointed out that an effective tester should correctly rank inbred lines for performance in hybrid combination, and that it should maximize the variance between testcross progeny to allow for efficient discrimination of new inbred lines. Consequently, lines with poor combining abilities are discarded and only good performing lines are advanced in the programme (Shahab et al., 2011). Valuable information regarding the performance of new lines with specific genetic background can be provided by using elite inbred testers but this only allows evaluation of new lines with alleles from a single genetic background (Packer, 2007). Alternatively, two inbreds can be used, but this would increase the cost of selection. However, the risk of discarding material that may combine well with germplasm adapted to a different region or genetic background than that of the tester can be increased by using single inbred line testers (Packer, 2007). Therefore two testers were used in the current study to discriminate inbred lines for yield over 5 environments.

1.10 The relationship between yield and secondary traits

A widely used successful method in plant breeding is selection; however, response to selection depends on many factors such as the interrelationship of the secondary traits (Geetha and Jayaraman, 2000; Jayakumar et al., 2007; Ilker, 2011). In the selection programs plant breeders' work with some yield components related to yield (Ilker, 2011; Raghu et al., 2011). However, Bello et al. (2010) asserts that breeding for high yield crops require information on the relationship of yield with other agronomic characters. Knowledge of the genetic association between traits is very useful for the establishment of selection criteria (Nastasic et al., 2010; Muhammad et al., 2011). This is because improving one trait might simultaneously change the expression of another trait due to their interrelationship. Maize grain yield is a quantitative trait in nature and controlled by many genes, thus, effective yield improvement and simultaneous improvement in yield components are

imperative (Geetha and Jayaraman, 2000; Bello and Olaoye, 2009; Srećkov et al., 2010). Similarly, Saidaiah et al. (2008) and Raghu et al. (2011) explained that grain yield is the end product of interaction among yield contributing components. Therefore, improving yield directly becomes difficult. Alteration in the expression of one trait is usually associated with a change in the expression of other traits (Ahmad and Saleem, 2003). The efficiency of selection in plant breeding can be improved by the knowledge of association between yield and its component traits and among the component parameters themselves (Bello et al., 2010; Raghu et al., 2011). This will clearly give an indication of which traits must be improved, which traits might be compromised and decisions made on what strategy is to be used to improve yield without compromising other important traits.

Selvaraj and Nagarajan (2011) found that plant height, ear height, ear length, and grain weight showed significant positive association with yield. On the other hand, days to tasseling and days to silking showed positive non-significant association with grain yield. These results were in agreement with Bello et al. (2010) who reported a positive and significant correlation between days to 50% tasselling with grain yield. However, they were in contrast with Muhammad et al. (2011) who reported a negative association between days to 50% silking and days to maturity. The discrepancy between the results is mainly due to different germplasm used and the environment, hence there is a need to evaluate genotypes under different environments to determine the effect of GXE on the correlation between yield and secondary traits.

1.11 Genotype by environment interaction in maize

The phenotype of an individual is determined by the genotype, environment and the interaction between genotype and environment (Martin, 2004). Genotype X environment interaction (GXE) causes complications in selecting hybrids for broad adaptation (Martin, 2004; Abdurahman, 2009; Babić et al., 2011). The relationship between phenotypic and genotypic values is impaired by the large GXE interaction (Ilker, 2011), hence the role of GXE interaction must be quantified in order to devise a breeding strategy. Genotype X Environment interaction is very important in sub-Saharan Africa because of fluctuation in environmental conditions, drought, low soil fertility, non-uniform management practices

and occurrence of diseases and pests (Martin, 2004). GXE is quantified by conducting multi-environment testing.

I. Crossing over interaction

Crossing over of genotypes is change in a genotype's rank from one environment to another (Crossa, 1990; Abdurahman, 2009). Crossa (1990) further explained that in crossing over, genotypic differences vary in direction among environments whereas; with non-crossing over genotypes reflects differences in magnitudes but not in direction. An appropriate stable cultivar which is capable of using resources that are available in high yielding environments, while maintaining above average performance in all other environments can also be identified (Nagabushan, 2008; Kandus et al., 2010). On the other hand, adaptability refers to the capacity of genotypes to give high yield under specific conditions. Cross over interaction delays the breeding progress as different sets of genotypes are selected in each environments (Abdurahman, 2009). Therefore there is a need to breed for genotypes with a wide adaptability to withstand different environmental conditions. Breeding for specifically adapted genotypes could also be an option; however, it is not durable because environmental conditions on the same locations change from year to year.

II. Analysis of Genotype X Environment interaction

Analysis of variance

Kandus et al. (2010) reported that combined ANOVA is frequently used to identify the existence of GXE interaction in multi-environmental experiments. Nonetheless, combined analysis has limitations that it assumes homogeneity of variance among environments required to determine differences among genotype differences (Kandus et al., 2010). Even though this analysis manage to determine the variance due to genotype, environment and the GXE interaction, it does not explore the response of the genotypes in the non-additive term (Kandus et al., 2010). Stability analysis is a tool that provides a general solution for the response of the genotypes to environmental change (Crossa, 1990; Kandus et al., 2010) .

Non-parametric test

The non-parametric statistics for GXE interaction based on ranks provide a useful alternative to parametric statistics, if the breeder is only interested in the existence of rank order differences over different environments (Martin, 2004). The rank order provides the breeder with the information of genotypes which are well ranked in all environments and those which are specifically well ranked in one environment. Principal component analysis (PCA) has more advantages than regression methods, because the regression method uses one statistic, the regression coefficient, to describe the pattern of response of a genotype across environments, and most of the information is wasted in accounting for deviations (Martin, 2004). On the other hand, PCA overcomes this difficulty by providing the scores on the PCA to describe the response pattern of genotypes (Crossa, 1990). The scores allows depicting GXE interactions into two dimensions (biplot) and identifying the factor responsible for the interaction (Abdurahman, 2009). The biplots provide a clear picture of genotypes and environments which are stable and the association between this. It also has the ability to group similar genotypes and environments in terms of stability. Crossa (1990) pointed out that the aim of principal analysis is to transform the data from one set of coordinate axes to another, which preserves as much as possible the original configuration of the set of points and concentrates most of the data structure in the first principal component axes. This analysis assumes that the original variables define a Euclidean space and similarity between individuals is measured as Euclidian distance (Crossa, 1990). As a result the structure of a two-way genotype-environment analysis data matrix is subspace of fewer dimensions (Crossa, 1990). Other methods can be used to group similar genotypes and environments, for example, Martin (2004) defined cluster analysis as a numerical classification technique that defines groups of clusters of individuals. There are two types of classification, non-hierarchical which assigns each item to a class and hierarchical groups which assigns the individuals into clusters and arranges these into a hierarchy for the purpose of studying relationships in the data (Crossa, 1990).

Additive Main effect and Multiplicative Interaction

The Additive Main effect and Multiplicative Interaction (AMMI) model encompasses several sources such as genotype main effect, environment main effect and the interaction with 0-F interaction's PCA axes (IPCA) and can thus be used to predict GXE (Crossa, 1990; Babić et al., 2011). Crossa (1990) mentioned that the AMMI model is used for model diagnosis to clarify GXE and to improve accuracy of yield estimates. Additionally, Babić et al. (2011) mentioned that the greatest benefit of AMMI is better understanding of genotypes, environments and the complex of their interactions. This basically helps in allocating genotypes to environments they are adapted to and in identifying the best environment for evaluation of genotypes. AMMI models can range from AMMI(1), AMMI(2) to AMMI(n), depending on the number of principal components used to study the interaction (Kandus et al., 2010). In the current study the AMMI-2 model was adopted.

1.12 Cultivar superiority and rank analysis

The stability of genotypes is studied by using simple and effective methods such as cultivar superiority and ranking methods. Lin and Binns (1988) defined a superior cultivar as one with a performance near the maximum in various environments. The genotypes are characterised with a parameter (*Pi*) by associating stability with productivity (Lin and Binns, 1988), in this way genotypes which are stable and high yielding can be identified. In other words this helps in identifying and separating genotypes with dynamic and homeostatic stability. Furthermore, cultivar superiority provides information on the general and specific adaptability of a genotype, whereas ranking method only provides information on generally good performers of the genotype across environments (Makanda, 2009). Thus, cultivar superiority is more useful because it measures both the performance and stability of the genotype. Basically *Pi* was defined as superiority index of the ith cultivar relative to the genotype with maximum performance in each environment and it is used to assess the superiority of the cultivar (Moremoholo and Shimelis, 2009). The smaller the value of *Pi*, the less is the distance to the genotype with maximum yield and the better the genotype.

1.13 Conclusion

Today the world is facing many challenges which include inadequate food production. This can be alleviated if different methods are integrated to improve the yield of maize especially in SSA. The biggest challenge is to bridge the gap between molecular breeding and conventional plant breeding. Molecular markers help in the study of genetic diversity which is crucial to ensure future progress in breeding. The study of genetic diversity helps in the identification of heterotic groups. Because when two inbred lines are crossed they may or may not exhibit heterosis. Generally inbred lines from different heterotic groups are more likely to exhibit heterosis, however, it is important to understand that the correlation between GD and heterosis is not always positive. Hence, the concept of combing ability is imperative. In addition, it is important to understand the nature of gene action operating for grain yield as this will help in developing effective breeding strategies. Moreover, grain yield is mainly influenced by the GXE interaction, which reduces heritability; hence, indirect selection is necessary. An ideal secondary trait for indirect selection must be genetically associated with grain yield under stress, highly heritable, cheap and fast to measure, stable within a measurable. Genotype X Environment interaction is very important in sub-Saharan Africa because of fluctuation in environmental conditions. Hence, this calls for a need to identify genotypes with high stability. For this purpose parametric methods like AMMI are adopted. In addition, non-parametric methods such as cultivar superiority index and mean ranks are used to quantify stability of genotypes because they are simple and effective methods.

1.14 References

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CHAPTER TWO

Assessment of Genetic Diversity among Maize Inbred Lines Using Molecular Markers and Phenotypic Traits

Abstract

The information on genetic diversity is essential for germplasm enhancement. The objectives of the study were therefore to analyse the genetic diversity present, compare molecular and morphological characteristics and define potential heterotic groups for the maize breeding program. Fifty maize inbred lines and 10 testers were studied to estimate the genetic variability at a phenotypic and molecular level using Euclidean distance determined with SSR markers. Thirty SSR primers resulting from 144 alleles were used to determine the genetic diversity of which 29 markers were amplified with average PIC of 0.55. There was a greater variation between testers and lines. The genetic distances ranged from 0.19 to 0.83 between lines, 0.29 to 0.83 between lines and testers and 0.26-0.86 among testers. On the basis of cluster analysis using SSR markers, the 60 lines were classified into two major groups and then further divided into seven sub groups which can be used to establish heterotic groups. The results showed that inbred lines in the same cluster were related by pedigree and origin. Furthermore, cluster analysis based on phenotypic data classified inbred lines into two major clusters and four sub-clusters. The results showed that inbred lines in the same group as well as in the sub-groups were similar in their physical and phenotypic characteristics. The clustering of genotypes using morphological data was similar to the clustering of genotypes using SSR markers for inbred lines and different in others depending on the number of traits used and heritability. Inbred lines clustered on the same group belonged to the same heterotic group while inbred lines on different clusters belonged to different heterotic groups. Hence, seven heterotic groups were identified based on SSR markers, while 3-4 heterotic groups could be inferred with phenotypic data. It is concluded that significant genetic diversity exists in the germplasm which is significant as a potential for producing superior genotypes.

Key words: Maize, Genetic Diversity, SSR Markers, Phenotypic Traits, Genetic Distance, Cluster Analysis, Heterotic Groups

2.1 Introduction

The information on genetic diversity is very important for germplasm enhancement (Hoxha et al., 2004; Shin et al., 2006; Dagne, 2008; Kumar et al. 2009; Makumbi et al., 2011). Frankham et al. (2002) defined genetic diversity as "the variety of alleles and genotypes present in a population, reflected in morphological, physiological and behavioral differences between individuals and populations". Assessing the levels and patterns of genetic diversity accurately is particularly helpful in maize. The data can be used for (i) maintenance and broadening of the genetic base of the elite germplasm; (ii) selection of appropriate parental lines for hybrid combinations; and (iii) generation of segregating progenies with maximum genetic variability for further selection (Prasanna et al., 2002). The knowledge obtained can be used to device new breeding strategies or to improve on the existing strategies. It is practically impossible to increase yield and other desirable characters of a crop if there is insufficient genetic diversity (Cholastova et al., 2011). This is because the selection of improved genotypes depend on the availability of genetic variability in the breeding material (Cholastova et al., 2011). Hence, It is imperative to know the extent of already existing genetic variability to improve genetic diversity of local germplasm (Cholastova et al., 2011). Maize does not contain all the desirable genes because of the restrictive genetic base that is being exploited by commercial breeders who are operating in the market. Li et al. (2002) reported that in China, the parentage of over 90 % of the hybrids consists of about 20 elite inbred lines. Similarly, the pedigrees of most hybrids in the United States are derivatives of 6–8 inbred lines (James et al., 2002; Rasmussen and Hallauer, 2006). Almeida et al. (2011) used SSR markers to analyse the genetic variability in populations of sweet corn, common corn and teosinte. The analysis of genetic diversity within populations of common corn and sweet corn showed low diversity (0.22 to 0.33), compared to the main populations of CIMMYT (International Maize and Wheat Improvement Center), which were analysed by SSR and showed values of 0.45 to 0.61. They concluded that maize populations may have limitations in future cycles of breeding. However, it is generally believed that genetic diversity might still be large in tropical germplasm because broad base varieties such as OPV's are still being grown.

The microsatellite or SSR markers can be used effectively in studying genetic diversity in maize. Simple Sequence Repeats (SSRs) are defined as sections of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species (Kumar et al., 2011). Powell et al. (1996) as cited by Kumar et al. (2009) reported that the term “microsatellite” was first coined by Litt & Lutty (1989). Microsatellite markers can either belong to the transcribed region or the non-transcribed region of the genome. However, there is rarely any information regarding their function. The SSR markers have a high degree of variability and thus are suited for population studies and to distinguish and identify closely related genotypes (Smith and Devey, 1994; Smith et al., 1997). This means that SSR markers can be used effectively to identify heterotic groups.

Grouping of similar germplasm is a first step in identifying promising heterotic patterns in the maize program as determined by genetic distance based on molecular markers (Legesse et al., 2008). Genetic distances (GD) between populations, individuals, or lines, whether estimated from co-ancestries or DNA fingerprints, have been widely used for descriptive analyses in crop plants. The examples of their application range from reconstructing breeding histories, describing patterns of genetic diversity, and assigning lines to heterotic or other biologically or economically important groups (Cheres et al., 2000). Such analyses are appealing because GD can be estimated without phenotyping the germplasm to be classified (Cheres et al., 2000). This is advantageous because phenotyping can be expensive and time consuming. The main multivariate technique used to measure GD is based on phenotypic characters (Bertan et al., 2007). Multivariate analysis is the major tool used in estimating GDs as it allows for the possibility of gathering many variables into one analysis (Bertan et al., 2007). Genetic distance can be estimated from various types of molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) (Semagn et al., 2012). However, the SSR markers are recommended ahead of the SNPs; because they are highly polymorphic (multiallelic) and can effectively be used to study genetic diversity (Ibitoye and Akin-Idow, 2010).

The most used statistics to estimate GD are Mahalanobis (D^2) and the Euclidean distance (Darbeshwar, 2000; Bertan et al., 2007). The data is then presented in a symmetrical matrix once the distance estimate between each genotype pair is obtained and the analysed by the use of a clustering/plotting procedure (Bertan et al., 2007). Pool observation can be separated into many subgroups to obtain homogeneity within and between the formed subgroups using the clustering method (Darbeshwar, 2000; Bertan et al., 2007). Plant Breeders can use hierarchical methods to group genotypes by a process that repeats itself at many levels, forming a dendogram without concern for the number of groups formed (Bertan et al., 2007). Different clustering methods can be used depending on the procedure that is most suitable to the data set. For example Tocher's clustering leads to the formation of one large cluster, whereas the UPGMA better discriminates the closer genotypes (Bertan et al., 2007). The data obtained from the clusters can be used to make inferences on the potential heterotic groups and new patterns that are needed to enhance yield in maize hybrids.

Heterotic group was defined by Melchinger and Gumber (1998) as "a group of related or unrelated genotypes from the same or different populations which show similar combining ability or heterotic response when crossed with genotypes from other genetically distinct germplasm groups". Heterotic patterns refer to specific pair of two heterotic groups which express high heterosis and consequently high hybrid performance in their cross. Hallauer and Miranda (1988) reported that hybrids created by crossing divergent inbred lines result in high hybrid vigour or expression of heterosis. This means that different heterotic groups can be formed based on the gene frequency of parental genetic materials employed to make crosses (Fato, 2010). When inbred lines are classified into heterotic groups, this will facilitate the exploitation of heterosis in maize, which can contribute to hybrid performance (Bidhendi et al., 2011).

The objectives of this study were as follows:

i) to analyse the genetic diversity present

ii) to define potential heterotic groups

iii) And attempt to identify the most representative testers for each potential heterotic group.

The information would be crucial in devising the breeding strategy for developing new hybrids in South Africa. Essentially the breeding objective was to enhance adaptation ability of the hybrids under the production culture in South Africa and SSA.

2.2 Materials and methods

2.2.1 Germplasm

Fifty (50) new maize inbred lines that were derived from subtropical x temperate population at the UKZN maize program (Table 2.2) and ten testers (Table 2.1) were selected for the genetic diversity study. These lines have shown productive potential in observation trials conducted during 2009/2010, 2010/11 and 2011/12 seasons across locations in Kwa-Zulu Natal and North West province. The maize inbred lines were derived using conventional breeding methods from many populations obtained from various sources ranging from narrow to broad base populations from Africa, USA and Asia. Temperate germplasm was also introgressed and incorporated into the tropical base germplasm collection to introduce the alleles for early maturing which are required to fit the germplasm in increasingly short seasons in Sub-Saharan Africa (SSA), and to facilitate late season planting, and to enhance standing ability and prolificacy to ensure adaptation under windy and low planting population conditions.

Table 2.1: List of maize inbred testers used in this study

No	Testers	Category	Country of origin
1	H24W	Regional	South Africa
2	I137TN	Regional	South Africa
3	PA-1	Regional	Zimbabwe
4	CML202	Regional	Zimbabwe
5	LP23	Regional	Mozambique
6	LP19	Regional	Mozambique
7	PA2	Regional	Zimbabwe
8	M162W	Regional	South Africa
9	MO17WX	International	United States of America
10	B73WX	International	United States of America

Table 2.2: List of inbred lines used in this study

Lines	Name	Lines	Name	Lines	Name	Lines	Name
1	DXL19	**16**	DXL59	**31**	DXL131	**46**	DMLF4-128
2	DXL24	**17**	DXL60	**32**	DXL136	**47**	DMLF4-157
3	DXL25	**18**	DXL61	**33**	DXL158	**48**	DMLF4-207
4	DXL34	**19**	DXL62	**34**	DXL161	**49**	DMLF4-214
5	DXL37	**20**	DXL68	**35**	DXL162	**50**	08CED6-7
6	DXL44	**21**	DXL69	**36**	DXL183		
7	DXL46	**22**	DXL98	**37**	DXL204		
8	DXL47	**23**	DXL101	**38**	DXL206		
9	DXL49	**24**	DXL106	**39**	DXL236		
10	DXL50	**25**	DXL108	**40**	DMSR-2		
11	DXL51	**26**	DXL112	**41**	DMSR-44		
12	DXL52	**27**	DXL116	**42**	DMLF4-8		
13	DXL54	**28**	DXL124	**43**	DMLF4-55		
14	DXL56	**29**	DXL126	**44**	DMLF4-85		
15	DXL58	**30**	DXL129	**45**	DMLF4-97		

2.2.2 Diversity analysis

The simple sequence repeat (SSR) method was used following the internal protocol.

Maize Samples

The 60 maize inbred lines were grown in a tunnel in four pots each, using pine bark medium in the University of KwaZulu-Natal. Leaf tissues were harvested from each of the four plants and the tissues were bulked. The leaf samples were stored at -20 C until use.

DNA extraction

Two leaf discs (punches or equivalent) approximately 20 mm diameters that were harvested from each plant were put in the specific well position. When the block is completed, a sheet of Air Pore Tape was put on the top of the block to seal. The block was then placed inside a plastic bag, together with 50g of silica gel and the material was then dried within 24 hours. The indicator silica gel was used to confirm if it was dehydrated (blue when it is dehydrated or pink when hydrated). The samples were then shipped to the DNA landmarks laboratory for genotyping.

SSR Primers

The use of the set of 30 SSR markers in this study was recommended by the Generation Challenge Program (GCP). The primer sequences of the 30 SSR markers are shown (Table 2.3). The marker phi046 did not amplify in PCR. The DNA quality was evaluated on 0.8% agarose gel (three columns per plate). Once the DNA quality passed the quality control, the DNA samples in the plate were diluted at 25ng ul^{-1}. The diluted DNA samples were then used for PCR amplification with the 30 SSR markers.

PCR amplification

PCR reactions were performed following the DNA landmarks internal protocol (PCR conditions depend on the type of marker). PCR amplification for marker nc130 was performed in a volume of 10µL containing approximately 1.0 µl Buffer PCR (10X) w $MgCl_2$ (15mM), 0 µl $MgCl_2$ (25mM), 1.0 µl dNTP (2mM), 5.0 µl Q solution (5X), 0.125 µl Primer F (10uM), 0.125 µl Primer R (10µM), 0.1 µl HotStar Taq polymerase (5U/µl), 1.0 µl DNA (~20ng/µl) and 1.65 µl H_2Odd. Reactions were run with an initial denaturation step for 15 minutes at 95°C, 1 minute at 55°C and 1 minute 72°C. This was followed by 35 cycles in 1 minute at 95°C, 55°C and 72°C then followed by a final extension at 72°C for 30 minute and 4°C forever. All the other PCR conditions for the markers had a total of 6 µl except for seven markers which had a total of 10 ul.

For the markers that are available in the primer stock, the forward primers were labelled with fluorescent dyes for detection on ABI 3730xl apparatus. For the new markers, the forward primers were tailed with M13 sequence and the M13 primer (cacgacgttgtaaaacgac) was labelled with one of the four fluorescence dyes (6FAM, PET, NED or VIC) for multiplexed PCR products detection using the ABI3730xl apparatus, the profile is shown (Figure 2.1). The allele sizes were scored using Gene Mapper software. Failed samples were repeated one to two times.

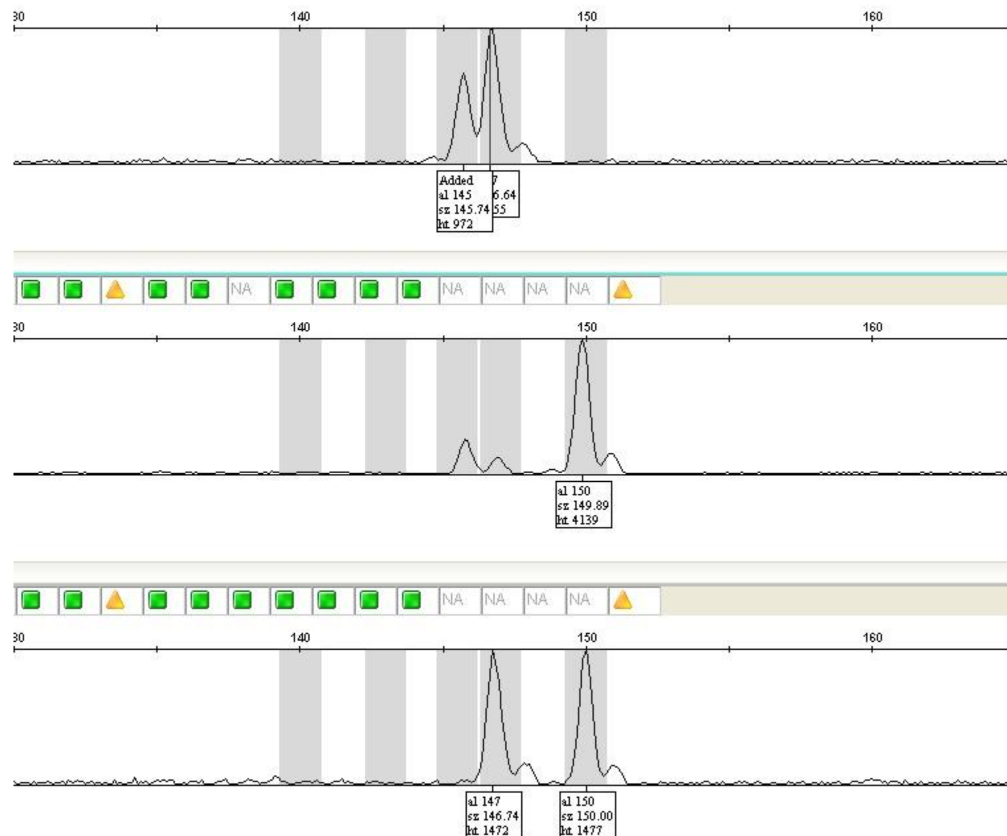

Figure 2.1: The profile of nc130

Statistical analysis

Genetic distances between pairs of lines were estimated according to the expression: GD = $1\text{-}GS_{ij}$, Where GS refers to Eucledian genetic similarity coefficient, between the lines *i* and *j.* The program GGT 2.0 (Van Berloo, 2007) was used to calculate the Eucledian distances between samples, the matrix of the genetic distances were used to create a UPGMA dendogram of the results.

Table 2.3: Microsatellite primer sequences used in the study

Marker	Chromosome	Motif	Forward Primer	Reverse Primer	Annealing temperature Tm
nc130	5	AGC	gCACATgAAgATCCTgCTgA	TgTggATgACggTgATgC	54
nc133		GTGTC	AATCAAACACACACCTTgCg	gCAAgggAATAAggTgACgA	
phi029	3	AGCG	TTgTCTTTCTTCCTCCACAAgCAgCgAA	ATTTCCAgTTgCCACCgACgAAgAACTT	56
phi031	6	GTAC	gCAACAggTTACATgAgCTgACgA	CCAgCgTgCTgTTCCAgTAgTT	60
phi041	10	AGCC	TTggCTCCCAgCgCCgCAAA	gATCCAgAgCgATTTgACggCA	56
phi046	3	ACGC	ATCTCgCgAACgTgTgCAgATTCT	TCgATCTTTCCCggAACTCTgAC	60
phi056	1	CCG	ACTTgCTTgCCTgCCgTTAC	CgCACACCACTTCCCAgAA	56
phi062	10	ACG	CCAACCCgCTAggCTACTTCAA	ATgCCATgCgTTCgCTCTgTATC	56
phi065		CACTT	AgggACAAATACgTggAgACACAg	CgATCTgCACAAAgTggAgTAgTC	
phi072	4	AAAC	ACCgTgCATgATTAATTTCTCCAgCCTT	gACAgCgCgCAAATggATTgAACT	56
phi075	6	CT	ggAggAgctCACCggCgCATAA	AAAggTTACTggACAAATATgC	54
phi076		GAGCGG	TTCTTCCgCggCTTCAATTTgACC	gCATCAggACCCgCAgAgTC	
phi079		CATCT	TggTgCTCgTTgCCAAATCTACgA	gCAgTggTggTTTCgAACAgACAA	
phi084	10	GAA	AgAAggAATCCgATCCATCCAAgC	CACCCgTACTTgAggAAAACCC	54
phi102228	3	AAGC	ATTCCgACgCAATCAACA	TTCATCTCCTCCAggAgCCTT	54
phi112		AG	TgCCCTgCAggTTCACATTgAgT	AggAgTACgCTTggATgCTCTTC	
phi114	7	GCCT	CCgAgACCgTCAAgACCATCAA	AgCTCCAAACgATTCTgAACTCgC	60
phi123		AAAG	ggAgACgAggTgCTACTTCTTCAA	TgTggCTgAggCTAggAATCTC	
phi227562	1	ACC	TgATAAAgCTCAgCCACAAgg	ATCTCggCTACggCCAgA	56
phi299852		AGC	gATgTgggTgCTACgAgCC	AgATCTCggAgCTCggCTA	
phi308707	6	AGC	gCAACAAgATCCAgCCgAT	gTCgCCCTCATATgACCTTC	54
phi331888		AAG	TTgCgCAAgTTTgTAgCTg	ACTgAACCgCATgCCAAC	
phi374118	3	ACC	TACCCggACATggTTgAgC	TgAAgggTgTCCTTCCgAT	56
phi96100	2	ACCT	AggAggACCCCAACTCCTg	TTgCACgAgCCATCgTAT	56
umc1161	8	GCTGGG	ggTACCgCTACTgCTTgTTACTgC	gCTCgCTgTTggTAgCAAgTTTTA	56
umc1304		TCGA	CATgCAgCTCTCCAAATTAAATCC	gCCAACTAgAACTACTgCTgCTCC	

Marker	Chromosome	Motif	Forward Primer	Reverse Primer	Annealing temperature Tm
umc1367	10	CGA	TggACgATCTgCTTCTTCAgg	gAAggCTTCTTCCTCgAgTAggTC	62
umc1545		AAGA	gAAAACTgCATCAACAACAAgCTg	ATTggTTggTTCTTgCTTCCATTA	
umc1917	1	CTG	ACTTCCACTTCACCAgCCTTTTC	ggAAAgAAgAgCCgCTTggT	52
umc2250	2	ACG	ACAggTCACAgATgTTCATCCAgg	CTCgACTggATCgCCTCCTC	58

2.2 3 Field experiment

Experimental design and management

Sixty inbred lines (50 experimental lines and 10 testers) were evaluated at Ukulinga Research Station (latitude = -29.66S, longitude= 30.40E, Altitude = 808 m.a.s.l) during the 2011/2012 season. The trial was planted on the 23rd of November 2011. The experiment was laid out in a Randomized Complete block design with two replications, the net plot length was 5 m, the distance between the stations was 0.3 m, and the distance between the rows was 0.9 m. After thorough land preparation, sowing was done by hand dibbling of seeds with two seeds per hill. Seedlings were thinned out three weeks after emergence to maintain single seedling per hill. A total of 250 Kg/ha (NPK) 2:3:4 (55 Kg/ha of N, 83 Kg/ha of P and 111 Kg/ha of K) was applied as basal fertilizer before planting and 250 kg/ha LAN (28% of N) was applied as a top dressing four weeks after crop emergence. Weeds were controlled by chemicals such as Basagran (to kill nutsedge), Gramoxone (all green weeds) and Troopers (broadleaf weeds including morning glory). The trials were rain-fed and harvesting was done by hand after the physiological maturity stage.

Data collection

The following traits were measured following standard protocols used at CIMMYT (Magorokosho et al., 2009):

a) **Plant height (cm):** measured as the distance between the base of a plant to the insertion point of the top ear. It was measured when all the plants had flowered in meters, since plants reach their maximum height at flowering.
b) **Ear height (cm):** measured as height from ground level up to the base of the upper most cobs bearing internode in meters.
c) **Ear position:** measured as the ratio of ear height to plant height. Small values indicate low ear position and large values will indicate high ear position.
d) **Days to pollination:** measured as the number of days after planting to 50% pollination.
e) **Root lodging:** measured as percentage of the plants per plot which have their stems inclining by more than 45°.
f) **Stem lodging:** measured as the percentage of plants per plot that have their stems broken below the ear.
g) **Tassel branch number:** measured as the number of primary branches.

h) **Leaf number:** measured as the number of leaves at flowering.

i) **Ear length:** measured from the base of the cob to the tip in cm.

j) **Grain yield:** measured as grain mass per plot adjusted to 12.5 % grain moisture and converted to tones per hectare.

k) **Grain texture:** rated on the scale from 1 (flint) to 5 (dent).

Data analysis

General analysis of variance was performed using GENSTAT (version 13th edition). Inbred lines data were analyzed as a Randomized Complete Block Design. This was done for each character according to the following model:

$$\mathbf{Y_{ij} = \mu + \beta_i + T_j + E_{ij}}$$

Where,

Y_{ij} = yield of genotype,

μ = grand mean,

β_i = Block effect of the jth block,

T_j = genotype effect of the ith treatment

E_{ij} = random error for the ith treatment in the jth block.

Estimation of genetic parameters

Genetic parameters were estimated for different traits on maize genotypes as follows:

Heritability (H^2)

Heritability in broad sense was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage (Darbeshwar, 2000).

$$H^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e/r} \text{ X } 100$$

Where, $\sigma^2 g$ = Genotypic variance, $\sigma^2 e$ = environmental variance and r= number of replications

2.3 Results

2.3.1 Polymorphism of SSR markers

The characteristics of the 29 SSR loci analysed are shown in Table 2.4. The number of alleles and their frequency at each locus was analysed to indicate polymorphism. A total of 114 alleles were observed in the 60 inbred lines with an average of 4.96 by using the 30 SSR markers of which 29 were amplified and one marker (phi046) not amplified in the PCR. The PIC for the SSR locus ranged from 0.29 (umc2250) to 0.81 (phi041) with an average of 0.55. Furthermore, heterozygosity in the inbred lines ranged from 0.35 to 0.75 with an average of 0.6).

Table 2.4: Characteristics of the 29 SSR loci analysed

SSR locus	No. alleles	PIC value	He
nc130	3.00	0.45	0.53
nc133	4.00	0.41	0.47
phi029	6.00	0.57	0.63
phi031	6.00	0.61	0.67
phi041	10.00	0.81	0.84
phi056	5.00	0.70	0.75
phi062	2.00	0.34	0.43
phi065	4.00	0.58	0.49
phi072	6.00	0.71	0.75
phi075	4.00	0.59	0.65
phi076	5.00	0.65	0.70
phi079	5.00	0.54	0.60
phi084	3.00	0.32	0.38
phi112	5.00	0.34	0.36
phi114	4.00	0.61	0.67
phi123	4.00	0.61	0.67
phi96100	8.00	0.72	0.75
phi102228	5.00	0.47	0.52
phi227562	7.00	0.66	0.71
phi299852	6.00	0.67	0.72
phi308707	4.00	0.57	0.64
phi331888	5.00	0.60	0.65
phi374118	3.00	0.39	0.49
umc1161	6.00	0.63	0.67
umc1304	7.00	0.60	0.66
umc1367	4.00	0.53	0.58
umc1545	5.00	0.65	0.70
umc1917	6.00	0.46	0.50
umc2250	2.00	0.29	0.35
Mean	4.97	0.55	0.60

2.3.2 Genetic distance among inbred lines

The genetic distances between the 50 lines and 10 testers are shown in Table 2.5. Genetic diversity of the 50 lines and 10 testers, respectively, was characterized by 29 SSR markers using Euclidean distance. The highest genetic distance between the lines was 0.83 and the lowest was 0.19. The highest genetic distance between the testers was 0.86 (MO17 and CML202) and the lowest was 0.26 (MO17 and B73WX). There was a greater variation between testers and lines. The highest GD above 0.8 was found between DXL101 and B73WX, DXL34 and LP23 and DXL112 and I137TN. Conversely, the lowest genetic distance, below 0.3 was found between DXL131 and I137TN, and DMSR44 and LP23 (Table 2.5).

Table 2.5: Genetic distance between lines and testers using molecular markers

Lines/Testers	H24W	I137TN	PA-1	CML202	LP23	LP19	PA-2	M162W	MO17	B73WX
DXL19	0.69	0.63	0.57	0.59	0.75	0.56	0.72	0.73	0.79	0.78
DXL54	0.48	0.49	0.65	0.63	0.63	0.70	0.67	0.47	0.60	0.47
DXL108	0.51	0.33	0.52	0.51	0.67	0.49	0.44	0.68	0.64	0.76
DXL183	0.57	0.56	0.56	0.58	0.61	0.53	0.64	0.65	0.61	0.75
DMSR44	0.60	0.61	0.62	0.65	0.29	0.58	0.71	0.69	0.58	0.80
DXL24	0.42	0.49	0.65	0.63	0.76	0.66	0.60	0.56	0.67	0.65
DXL56	0.61	0.52	0.52	0.59	0.50	0.43	0.53	0.61	0.53	0.61
DXL112	0.61	0.81	0.67	0.66	0.72	0.71	0.59	0.67	0.59	0.73
DXL204	0.56	0.56	0.61	0.62	0.62	0.48	0.62	0.67	0.59	0.77
DXL25	0.43	0.57	0.68	0.54	0.77	0.67	0.61	0.62	0.67	0.69
DXL58	0.54	0.57	0.65	0.44	0.61	0.52	0.57	0.65	0.64	0.72
DXL116	0.56	0.46	0.61	0.47	0.60	0.55	0.57	0.62	0.53	0.68
DXL206	0.53	0.52	0.54	0.59	0.62	0.53	0.59	0.58	0.56	0.59
DMLF48	0.39	0.55	0.61	0.63	0.73	0.60	0.66	0.55	0.53	0.64
DXL34	0.46	0.70	0.71	0.70	0.80	0.70	0.67	0.65	0.64	0.62
DXL59	0.47	0.57	0.56	0.42	0.55	0.56	0.65	0.71	0.39	0.80
DXL236	0.47	0.38	0.59	0.36	0.61	0.48	0.61	0.66	0.50	0.70
DMLF455	0.43	0.47	0.59	0.51	0.64	0.55	0.57	0.65	0.51	0.72
DXL37	0.48	0.59	0.65	0.63	0.77	0.61	0.63	0.62	0.60	0.68
DXL60	0.37	0.57	0.73	0.54	0.65	0.68	0.79	0.48	0.61	0.63
DXL124	0.51	0.53	0.56	0.54	0.57	0.61	0.64	0.68	0.70	0.83
DMLF485	0.35	0.52	0.55	0.66	0.75	0.54	0.47	0.64	0.56	0.64
DXL44	0.44	0.65	0.63	0.76	0.79	0.62	0.62	0.61	0.62	0.60
DXL61	0.32	0.51	0.57	0.69	0.66	0.59	0.52	0.64	0.48	0.64
DXL126	0.51	0.56	0.71	0.60	0.60	0.58	0.67	0.53	0.47	0.68
DMLF497	0.49	0.57	0.65	0.61	0.57	0.61	0.48	0.65	0.54	0.76
DXL46	0.54	0.62	0.61	0.69	0.63	0.61	0.57	0.53	0.66	0.64

Lines/Testers	H24W	I137TN	PA-1	CML202	LP23	LP19	PA-2	M162W	MO17	B73WX
DXL62	0.49	0.54	0.47	0.52	0.61	0.50	0.52	0.71	0.68	0.80
DXL129	0.51	0.63	0.62	0.70	0.61	0.58	0.51	0.65	0.44	0.77
DMLF4128	0.48	0.42	0.65	0.50	0.57	0.55	0.47	0.62	0.70	0.68
DXL47	0.47	0.57	0.44	0.58	0.74	0.62	0.48	0.57	0.74	0.62
DXL68	0.42	0.36	0.52	0.40	0.60	0.52	0.53	0.65	0.60	0.75
DXL131	0.37	0.29	0.54	0.59	0.68	0.51	0.52	0.58	0.43	0.63
DMLF4157	0.43	0.50	0.52	0.51	0.61	0.45	0.48	0.57	0.57	0.61
DXL49	0.43	0.50	0.62	0.67	0.80	0.61	0.51	0.39	0.57	0.34
DXL69	0.56	0.34	0.57	0.63	0.66	0.59	0.49	0.46	0.63	0.57
DXL136	0.46	0.37	0.46	0.54	0.67	0.49	0.44	0.68	0.64	0.72
DXL50	0.38	0.46	0.67	0.54	0.69	0.56	0.57	0.51	0.63	0.52
DXL98	0.51	0.43	0.59	0.57	0.64	0.47	0.38	0.65	0.70	0.69
DXL158	0.53	0.53	0.55	0.63	0.63	0.55	0.37	0.68	0.77	0.75
DMLF4207	0.49	0.63	0.74	0.73	0.61	0.67	0.61	0.49	0.39	0.59
DXL51	0.49	0.59	0.68	0.67	0.74	0.61	0.64	0.39	0.57	0.48
DXL101	0.53	0.44	0.63	0.40	0.52	0.54	0.65	0.75	0.59	0.83
DXL161	0.53	0.66	0.65	0.63	0.57	0.54	0.60	0.62	0.40	0.75
DMLF4214	0.41	0.57	0.68	0.64	0.74	0.61	0.57	0.57	0.54	0.66
DXL52	0.48	0.43	0.58	0.50	0.72	0.60	0.59	0.67	0.69	0.70
DXL106	0.55	0.50	0.50	0.61	0.61	0.46	0.57	0.71	0.57	0.79
DXL162	0.61	0.68	0.69	0.62	0.75	0.74	0.68	0.63	0.68	0.73
DMSR2	0.58	0.56	0.64	0.53	0.37	0.63	0.66	0.64	0.50	0.70
08CED67	0.34	0.51	0.65	0.61	0.70	0.58	0.52	0.57	0.52	0.59
Mean	0.49	0.53	0.60	0.58	0.65	0.57	0.58	0.61	0.59	0.68
Max	0.69	0.81	0.74	0.76	0.80	0.74	0.79	0.75	0.79	0.83
Min	0.32	0.29	0.44	0.36	0.29	0.43	0.37	0.39	0.39	0.34

The genetic distances between the 50 lines and 10 testers using phenotypic traits are shown in Table 2.6. The highest genetic distance between the lines was 0.43 and the lowest was 0.03. The highest genetic distance between the testers was 0.27 (B73WX and T10) and the lowest was 0.009 (CML202 and PA-1). The highest GD of 0.3 was found between T10 and DXL101. Conversely, the lowest genetic distance of 0.00 was found between DMSR-2 and PA-1 (Table 2.6).

Table 2.6: Genetic distance between the lines and testers using phenotypic traits

Lines/Testers	HA24W	PA-1	CML202	LP23	LP19	T9	T11	T10	PA-2
DXL98	0.11	0.02	0.02	0.10	0.04	0.07	0.03	0.17	0.03
DXL69	0.19	0.03	0.05	0.18	0.16	0.12	0.05	0.16	0.12
DXL68	0.19	0.03	0.04	0.06	0.04	0.09	0.06	0.23	0.08
DXL62	0.20	0.04	0.04	0.12	0.07	0.13	0.04	0.24	0.07
DXL60	0.10	0.02	0.02	0.12	0.03	0.06	0.03	0.19	0.02
DXL59	0.09	0.02	0.04	0.03	0.03	0.17	0.09	0.19	0.03
DXL58	0.17	0.10	0.05	0.16	0.16	0.20	0.03	0.31	0.13
DXL56	0.10	0.04	0.04	0.13	0.14	0.14	0.05	0.16	0.09
DXL54	0.19	0.02	0.08	0.19	0.11	0.06	0.08	0.12	0.11
DXL52	0.12	0.02	0.06	0.11	0.10	0.07	0.02	0.13	0.08
DXL51	0.27	0.04	0.23	0.24	0.14	0.04	0.09	0.12	0.16
DXL50	0.19	0.05	0.16	0.21	0.11	0.02	0.10	0.09	0.13
DXL49	0.11	0.28	0.22	0.17	0.19	0.11	0.22	0.11	0.20
DXL47	0.07	0.05	0.05	0.05	0.05	0.13	0.09	0.16	0.05
DXL46	0.04	0.02	0.03	0.07	0.02	0.02	0.04	0.10	0.01
DXL44	0.07	0.07	0.07	0.03	0.06	0.17	0.12	0.20	0.07
DXL37	0.09	0.05	0.05	0.05	0.06	0.14	0.09	0.18	0.07
DXL34	0.12	0.20	0.15	0.11	0.21	0.29	0.22	0.29	0.19
DXL25	0.09	0.15	0.10	0.08	0.12	0.23	0.18	0.25	0.12
DXL24	0.06	0.11	0.08	0.05	0.08	0.17	0.16	0.20	0.09
DXL236	0.06	0.02	0.03	0.05	0.04	0.19	0.07	0.20	0.02
DXL206	0.08	0.02	0.05	0.02	0.04	0.18	0.11	0.22	0.04
DXL19	0.10	0.22	0.16	0.21	0.25	0.22	0.18	0.27	0.23
DXL183	0.08	0.02	0.06	0.05	0.06	0.18	0.09	0.20	0.05
DXL162	0.14	0.03	0.06	0.06	0.03	0.14	0.08	0.19	0.05
DXL161	0.06	0.18	0.12	0.13	0.12	0.22	0.16	0.21	0.12
DXL158	0.02	0.16	0.11	0.10	0.14	0.24	0.15	0.21	0.09
DXL136	0.09	0.04	0.05	0.13	0.10	0.08	0.08	0.11	0.07
DXL131	0.06	0.03	0.06	0.07	0.09	0.18	0.07	0.18	0.05
DXL129	0.09	0.01	0.03	0.05	0.06	0.13	0.03	0.16	0.04
DXL126	0.14	0.18	0.18	0.12	0.16	0.10	0.12	0.15	0.18
DXL124	0.08	0.05	0.08	0.10	0.03	0.12	0.10	0.14	0.03
DXL116	0.10	0.14	0.11	0.15	0.15	0.12	0.12	0.14	0.14

Lines/Testers	HA24W	PA-1	CML202	LP23	LP19	T9	T11	T10	PA-2
DXL112	0.09	0.11	0.08	0.12	0.13	0.11	0.10	0.13	0.12
DXL108	0.07	0.05	0.05	0.06	0.07	0.09	0.06	0.13	0.07
DXL106	0.13	0.06	0.03	0.14	0.08	0.12	0.02	0.17	0.05
DXL101	0.18	0.10	0.08	0.14	0.10	0.28	0.16	0.30	0.09
DMSR-44	0.05	0.05	0.06	0.01	0.04	0.17	0.11	0.21	0.05
DMSR-2	0.07	0.00	0.04	0.03	0.02	0.15	0.07	0.21	0.02
DMLF4-97	0.08	0.17	0.14	0.12	0.16	0.12	0.17	0.13	0.14
DMLF4-85	0.11	0.07	0.10	0.14	0.09	0.04	0.09	0.07	0.08
DMLF4-8	0.03	0.14	0.09	0.09	0.10	0.14	0.15	0.13	0.09
DMLF4-55	0.05	0.18	0.14	0.08	0.11	0.17	0.19	0.18	0.11
DMLF4-214	0.15	0.12	0.11	0.11	0.16	0.22	0.16	0.26	0.15
DMLF4-207	0.05	0.08	0.05	0.08	0.09	0.10	0.08	0.10	0.07
DMLF4-157	0.11	0.07	0.08	0.15	0.12	0.06	0.06	0.08	0.10
DMLF4-128	0.15	0.16	0.15	0.17	0.17	0.08	0.15	0.11	0.17
Mean	0.11	0.09	0.09	0.11	0.10	0.14	0.10	0.18	0.09
Max	0.27	0.28	0.23	0.24	0.25	0.29	0.22	0.30	0.23
Min	0.02	0.00	0.02	0.01	0.02	0.02	0.02	0.07	0.01

2.3.3 Cluster analysis based on molecular markers

The genotypes are grouped into two major clusters I and II, whereby, cluster I is the largest and cluster II has eight genotypes (Figure 2.2). They are further divided into seven clusters (A, B, C, D, E, F and G) in which cluster A is the largest (21 genotypes), followed by cluster D (14 genotypes), cluster B (13 genotypes), cluster F (4 genotypes), cluster G (4 genotypes) and cluster C (2 genotypes) is the smallest. Group A was further subdivided into seven sub-clusters (A_1, A_2, A_3, A_4, A_5, A_6 and A_7). Additionally, group B (B_1, B_2, B_3 and B_4) and D (D_1, D_2 and D_3) were further subdivided into four and three sub-clusters respectively.

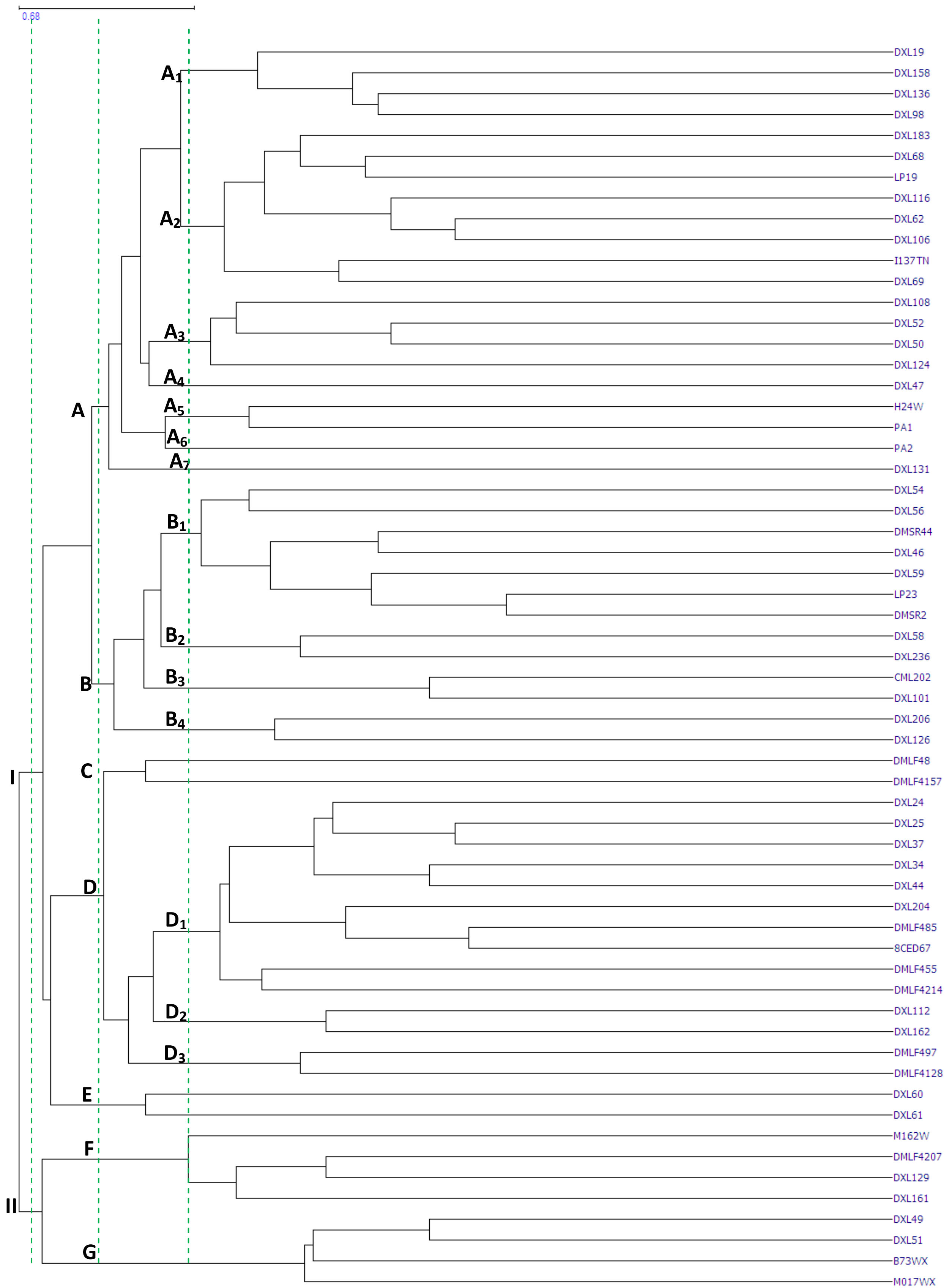

Figure 2.2: Dendogram of 60 maize inbreds based on 29 SSR molecular marker data using Euclidean distance

2.3.4 Heritability of phenotypic traits

Generally, many traits in this study exhibited high heritability. Plant height, number of tassel branches, percentage of prolific plants and grain texture had heritability above 80%. Anthesis date, ear position, stem and root lodging had the lowest heritability (Table 2.7).

Table 2.7: Genetic parameters of grain yield and secondary traits

Genetic parameters	Grain yield (t ha^{-1})	Plant height (cm)	Ear height (cm)	Ear position	Leaf number	No. of tassel branch
σ^2G	8.74 x 10^{-4}	489.60	212.30	2.0 x 10^{-3}	1.26	19.77
σ^2E	9.32 x 10^{-4}	227.20	547.40	1.60 x 10^{-2}	2.28	9.53
σ^2P	1.81 x 10^{-3}	716.80	759.70	1.82 x 10^{-2}	3.54	29.29
H^2 (%)	65.23	81.17	43.68	21.41	52.53	80.58
Mean	0.12	175.52	79.77	0.45	11.59	11.77
Genetic parameters	**Stem lodging (%)**	**Root lodging (%)**	**% of prolific plants**	**Anthesis date**	**Ear length (cm)**	**Grain texture**
σ^2G	11.10	368.00	8.74	0.00	1.62	1.08
σ^2E	375.00	3731.00	3.86	29.90	1.07	0.43
σ^2P	386.10	4099.00	12.60	29.53	2.69	1.50
H^2 (%)	5.59	16.48	81.92	0.00	75.23	83.50
Mean	4.10	35.65	3.89	75.36	13.20	2.72

σ^2G= Genotypic variance, σ^2E = Environmental variance, σ^2p = Phenotypic variance, H^2=Broad sense heritability

2.3.5 Cluster analysis based on morphological data

The dendogram of 60 maize inbred lines of all morphological data (all 12 traits) is shown in Figure 2.3. There are two major Groups (I and II), in which Group II is the largest with one inbred line in Group I. At 0.7 cut-off point three Groups are identified (A, B and C), in which Group B and C are further subdivided into two sub-clusters (B_1 and B_2, C_1 and C_2, respectively) at 0.8 cut-off point.

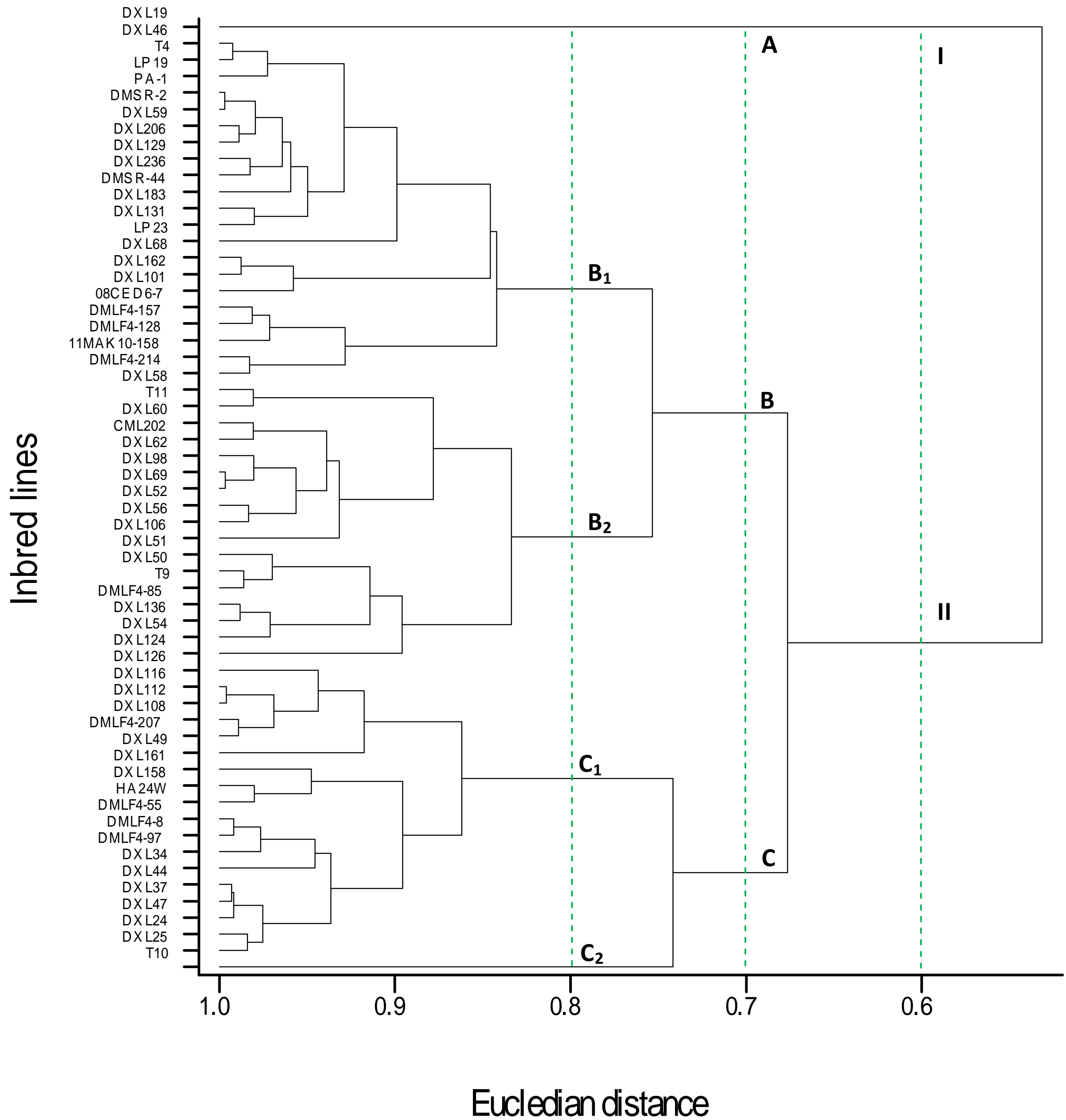

Figure 2.3: Dendogram based on 12 phenotypic traits of 60 maize inbred lines

The dendogram of 60 maize inbred lines based on morphological data (traits with heritability above 40%) is shown in Figure 2.4. There are two major Groups (I and II) which constitute approximately the same number of genotypes. At 0.7 cut-off point three Groups are identified (A, B and C), in which all Groups are further subdivided into two sub-clusters (A_1 and A_2, B_1 and B_2 and C_1 and C_2 respectively) at 0.8 cut-off point.

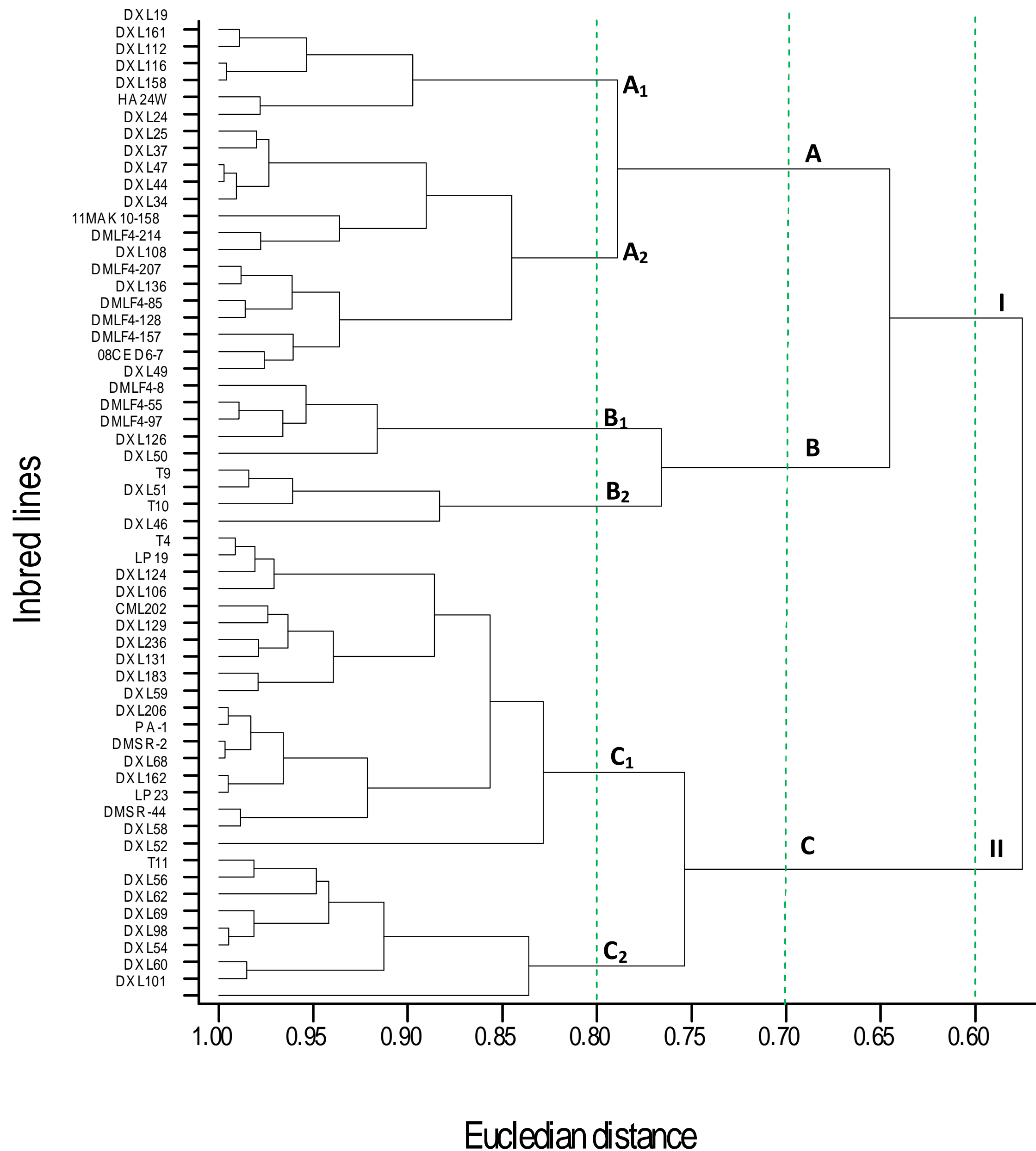

Figure 2.4: Dendogram of 60 maize inbreds based on 7 moderate to high heritable phenotypic traits (above 40%)

The dendogram of 60 maize inbred lines based on morphological data (highly heritable traits) is shown in Figure 2.5. Highly heritable traits (plant height, grain texture, percentage of prolific plants and number of tassel branch) were selected to construct a dendogram. There are two major clusters at a 0.6 cut-off point; however, five clusters (A, B, C, D and E) are displayed at a cut-off point of 0.7. Furthermore, at 0.8 cut-off point, cluster C is sub-divided into C_1 and C_2 and cluster B is sub divided into two groups (B_1 and B_2).

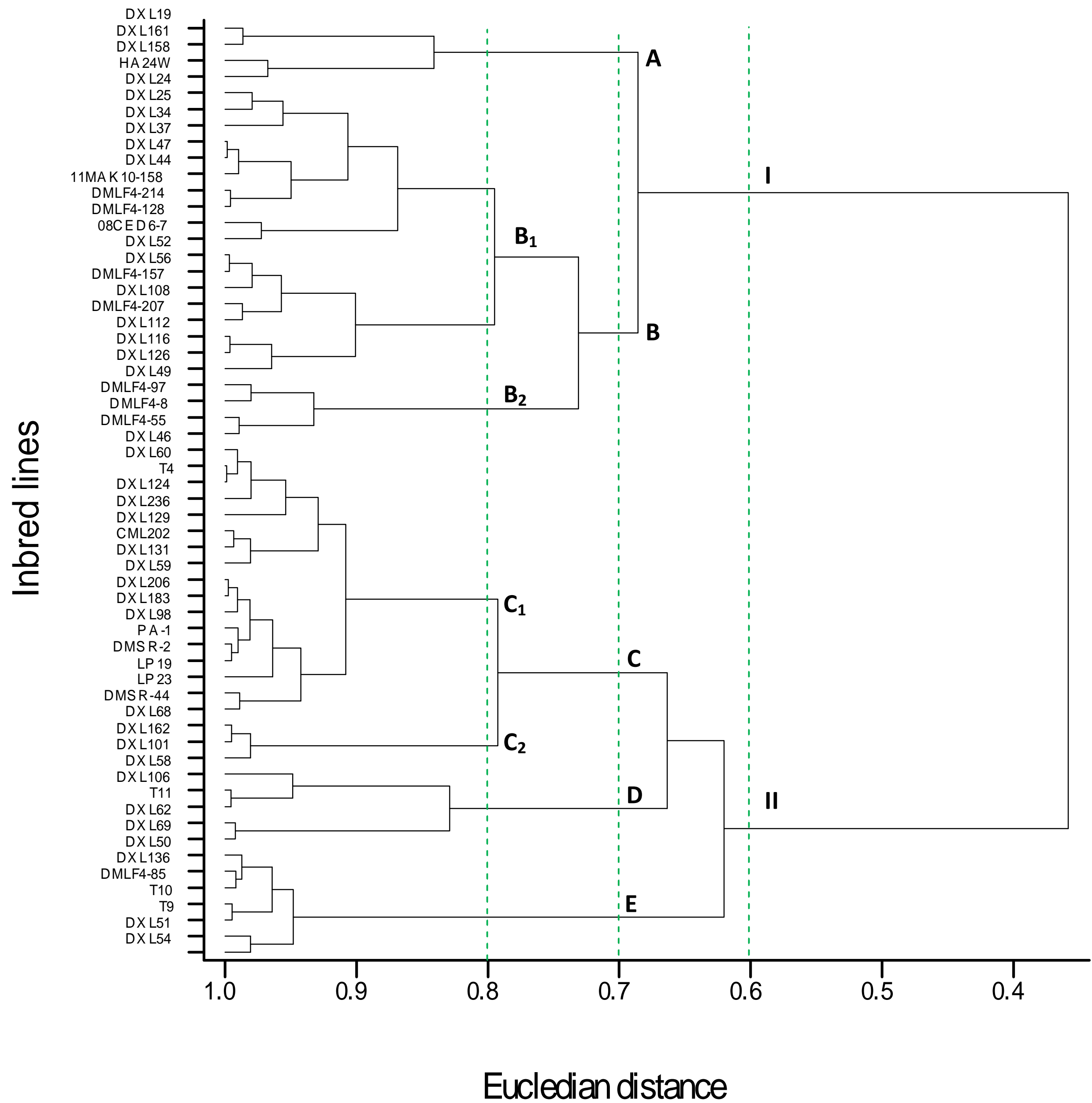

Figure 2.5: Dendogram of 60 maize inbreds based on 4 highly heritable (>80%) phenotypic traits

Five testers were selected to correlate the genetic distance between lines and testers using molecular markers and the genetic distance between lines and testers using phenotypic traits. The correlation was positive and significant for tester CML202 (0.29), LP19 (0.28) and it was positive and non-significant for LP23 (0.22) and B73WX (0.22). However, the correlation was negative and non-significant for PA-1 (0.03).

2.4 Discussion

2.4.1 Polymorphism of SSR markers

Markers in this study were polymorphic except for one marker. The average number of alleles observed for the loci was moderate (4.96). This showed that the SSR markers were informative and had the potential to detect differences among the inbred lines based on their genetic relationships. The results are in contrast with Hoxha et al. (2004) who reported mean number of alleles of 9.1 with 20 SSR markers in 20 Albanian local maize populations and Yao et al. (2008) who reported an average of 6.4 alleles per locus with 45 SSR markers in 124 maize landraces. This indicates that the genetic base in the current study was moderate compared to previous studies. However, Kostova et al. (2006) found a low average of 1.9 per locus for two B37 populations. The number of PIC for the SSR locus exhibited an average of 0.55, a value close 0.6 reported by Li et al. (2002). However, Legesse et al. (2007) reported a lower value of 0.33. Moreover, Gissa (2008) elucidated that the discrepancy in the number of alleles between studies could be explained mainly due to the size of the samples studied; expected diversity or uniformity based on pedigrees, and most importantly, repeat type of SSR used. Heterozygosity in the inbred lines ranged from 0.35 to 0.75 with an average of 0.6, indicating high levels of polymorphism in the inbreds. In contrast, Kostova et al. (2006) reported a low average heterozygosity of 0.170 for a maize population from MRI-Kneja and 0.046 for populations from IFC - Pleven. The differences in the estimates of genetic distances could be due to the fact that in the current study, inbreds used were derived from a diverse germplasm as temperate germplasm was incorporated into tropical germplasm.

2.4.2 Genetic distance between lines

There was a greater variation between testers and lines. DXL101 and B73WX, DXL34 and LP23 and DXL112 and L137TN, were found to be the most distantly related lines, this indicates that these lines belong to different heterotic groups. In addition, they have different gene frequency. Hence, have the potential to produce superior hybrids when crossed. This is consistent with Hallauer and Miranda (1988) who stated that the genetic

divergence of parental varieties determines the manifestation of heterosis and the heterotic patterns determines the genetic divergence of two parental varieties. Conversely, the lowest genetic distance was found between DXL131 and L137TN and DMSR44 and LP23, indicating that they were most closely related to each other with similar gene frequency. Therefore, they belong to the same heterotic group and have a less potential to produce superior hybrids. This is because crossing lines with similar gene frequency results in inbreeding depression rather than hybrid vigour.

2.4.3 Cluster analysis based on molecular data and phenotypic data

SSR markers

Inbred line clustered in group A_1 (DXL19) has a Kenya background, and the other inbred lines in this group (DXL 158 and DXL136) are derived from the same population. Inbred lines (DMSR2 and DMSR44) in group B_1 share a common parent LP23 hence, they are grouped together. This shows that these two lines inherited most of the genes from this particular parent. Similarly, inbred lines in sub-cluster B_2 (DXL58 and DXL236) share a common parent I137TN, however, this parent is clustered in sub-cluster A_2. This therefore, reveals that the two inbred lines did not inherit most genes from this parent but from the other parents that were used in the pedigree cross. In addition, inbred lines in sub-cluster B_3 (CML202 and DXL101), are put together because DXL101 has a CML202 background. DXL206 and DXL126 in sub-cluster B_4 have a CIMMYT background. Furthermore, inbred lines in Group D sub-cluster D_1 (DXL24, DXL25, DXL37, DXL34 and DXL44), are all temperate lines that were derived from a subtropical x temperate populations. Moreover, the DML lines grouped in cluster D_1 and D_3 all share a common parent 08CED67. However, DMF4207 are clustered in Group F, DMLF48 and DMLF157 clustered in Group C. DXL112 and DXL162 clustered in sub-cluster D_2 both have CML445 as one of the parent. DXL129 and DXL161 are sub-clustered in group F because they are from the same population. In Group G, B73WX and MO17WX are put together because they possess USA background and are the temperate lines.

All 12 phenotypic traits

Most of the inbred lines in Group A were short, with low ear placement, ranging from low to high leaf number. They were non-prolific, late, small ear length and flint. DXL19 was found

alone because it had 100% stem and root lodging. This is because DXL19 possess tropical Kenya background and as a result is not adaptable to the South African environment. The results are also confirmed by the molecular data as it was very different from the other three inbred lines it was grouped with. Inbred lines in Group B had low ear placement, low leaf number, moderate tassel branch number, moderate stem lodging, non-prolific, early and dent. Six of the inbred lines in Group B_1 were put on the same cluster (A) in the dendogram using molecular markers. Further, inbred lines in Group C were taller, high tassel branch number, prolific, long ears, moderate stem lodging, moderate flowering date, semi-dent and semi-flint. About 11 inbred lines in Group C were grouped similarly as in molecular markers, the TAB lines and down mildew lines (DML) lines. However, the DML inbred lines were not all grouped together just as in the molecular data.

Phenotypic traits with heritability greater than 40%

Inbred lines in Group A_1 were prolific, moderate ears, high tassel branch number, moderate ear position, moderate ear height. Inbred lines in Group A_2 were low yielding, low ear position, low leaf number, low tassel branch number, low stem lodging, semi-flint and semi-dent. In addition, nine of the inbred lines in Group A are also grouped together in Group D by molecular markers. Inbred lines in Group B were tall, high ear height, moderate ear position, moderate to high leaf number, moderate tassel branch, prolific and dent. DML lines are put together in Group A and extended to Group B, sub-cluster B_1 just as in molecular data. Moreover, inbred lines in Group C, sub-cluster C_1 were short to moderate, low ear height, small tassel branch numbers, high leaf number, highly susceptible to stem and root lodging, moderate prolificacy, early, low ear length and flint. Inbred lines in Group C_1 are temperate lines. Moreover, DMSR2 and DMSR44 were put together with one of their parents LP23 in cluster C_1 just as in the molecular data in cluster B1. Inbred lines in Group C, sub-cluster C_2 were moderate to short, low ear height, low leaf number, low stem lodging, moderate root lodging, high ear length and dent.

Phenotypic traits with heritability greater than 80%

Inbred lines in Group A were prolific, high tassel branch number, susceptible to stem and root lodging, prolific and dent. Inbred lines in Group B_1 were low yielding, moderate to low plant height, low ear position, moderate stem and root lodging, prolific, semi-flint and semi-

dent. Inbred lines in Group B_2 were tall, prolific, semi-dent and semi-flint and had moderate tassel branch. Ten of the inbred lines in Group B were similarly grouped together in Group D by molecular markers. Furthermore, all DTAB lines and DML lines, except for one DML line are put together in Group B as in molecular markers. Inbred lines in C had moderate yield, high leaf number, high stem and root lodging, moderate prolificacy, short ears, and dent grain. Five of the inbred lines in Group C are grouped together in Group B of the molecular data. Inbred lines in Group D had high yield, short, long ears, moderate leaf number, moderate root lodging and flint grain.

Clustering of inbred lines using phenotypic data was similar and different in some aspect to clustering using the 29 SSR markers. The correlation between GD for molecular markers and phenotypic traits was positive but not strong; this could be attributed to the genotype X environment interaction on the morphological data. These results are similar to Anas and Yoshida (2004) who also reported a positive correlation between the GD of molecular data and phenotypic data. Future studies will employ many sites to reduce the effects of GXE on masking genetic differences between the lines. The results are in line with Gissa (2008) who reported that in some cases, lines tightly clustered together were closely related by pedigree using phenotypic data. In addition, Cholastova et al. (2011) explained that phenotypic markers failed to detect the differences in closely related genotypes and elite breeding germplasm. Consequently, biochemical and cytological markers are used to monitor germplasm biodiversity. Moreover, in the current study clustering of genotypes using highly heritable traits was different and similar in some aspect to clustering of genotypes using all the traits. This is because the highly heritable traits were less influenced by the environment. The results are in agreement with, Anas and Yoshida (2004) who studied the relationship between phenotypic performance and genetic diversity determined by SSR markers and found that the grouping of genotypes based on SSR markers was similar to the grouping based on the combination of highly phenotypic heritable traits. Additionally, Kumar et al. (2009) reported a strong correlation between morphological genetic distance and SSR genetic distance. However, in the current study when the number of traits was reduced the accuracy of results was also reduced hence, it is better to use many traits with high heritability to get the best results. Furthermore, Gissa (2008) reported that since some similar inbred lines related by pedigree based on morpho-agronomic data were grouped

together, indicates that the morpho-agronomic traits can be used at least for primary characterization of maize inbred lines.

2.4.4 Potential heterotic grouping

Inbred lines were extremely different in various clusters due to differences in origin and pedigree; this confirms the presence of genetic diversity available. The inbred lines which are in different clusters can be assigned to different heterotic groups and the ones on the same cluster can be allocated in the same heterotic group. The molecular marker data grouped inbred lines in accordance with their origin and pedigree, which confirmed the reliability of using SSR markers for genetic diversity studies. The results are in agreement with Reif et al. (2003) who concluded that there is an excellent agreement between relationships obtained by SSR analyses and pedigree information. Similarly, Aguiar et al. (2008) did a study to determine heterotic groups of germplasm lines of tropical maize by test crosses and by SSR markers and to compare five grouping methods of heterogeneous maize. They found that grouping by SSR markers was consistent with the genealogy of the lines and is a useful procedure for the formation of heterotic groups of tropical maize lines. Therefore, SSR markers can be complemented with phenotyping in field trials to identify heterotic groups and to introgress exotic germplasm (Reif et al., 2003). Barata and Carena (2006) reported that groups of genetically similar germplasm using SSR markers could not be identified accurately and reliably even when the available germplasm was diverse. They concluded that extensive field evaluation is recommended to classify unrelated inbred lines of maize. In the present study, seven heterotic groups have been identified (A, B, C, D, E, F and G) using SSR markers. Both molecular and phenotypic data indicate clearly that heterotic grouping can be simplified into two broad Groups I and II, or further expanded to 3, 4 or 7 groups. Importantly results indicate that the resources are limiting good phenotypic data and accurate pedigree information could be used as bases to form clusters or heterotic groups.

2.5 Conclusion

From the study it can be concluded that,

- High diversity in the inbred lines implies that there is a high potential for producing superior hybrids. This indicates that the introgression of exotic germplasm was successful at broadening the diversity in the program.
- Inbred lines were clustered according to the existing pedigree information and origins of the lines confirming that maintenance of accurate records is crucial in fixing "heterotic" clusters.
- Clustering of inbred lines using morphological data was effective; however, evaluating genotypes in multi environments could make the method even more accurate and reliable; because the discrimination power increased with increasing heritability of traits.
- SSR markers were more effective in clustering genotypes that morphological data
- Seven heterotic groups have been identified (A, B, C, D, E, F and G) using SSR markers, whereas analysis of phenotypic traits revealed four potential heterotic groups

This study has implications for breeding that appropriate parental lines for hybrid combinations can be selected from the clusters, and crosses can be made between the clusters, and between sub-clusters within the broad clusters. Genetic diversity can be maintained by making new pedigree crosses within identified clusters.

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CHAPTER THREE

Assessment of Maize Testcrosses for Cultivar Superiority

Abstract

Selection of superior cultivars is complicated by the presence of significant genotype X environment (GXE) interaction effects. This is one factor that delays the breeding progress as it affects the ranking of hybrids from one environment to another. The objectives of the study were to assess the level of genotype X environment interaction and cultivar superiority of the new maize germplasm lines. One hundred and ninety hybrids were planted in five environments comprising three locations and two seasons. The hybrids were laid out in an incomplete block design with two replications. Data were analysed using the REML tool in GenStat 14th edition. The AMMI statistical model was used to describe GXE Interaction and adaptation. The results revealed significant differences between hybrids and environments as main effects and their interaction. Only the IPCA1 and IPCA2 were significant, hence the AMMI-2 model was adopted. The results revealed that Cedara in 2009/10 was the highest yielding (5.51 t ha^{-1}) environment and Ukulinga in 2011/12 was the lowest yielding (1.49 t ha^{-1}) environment. The hybrid GMH113 was the most adaptable genotype in all environments, but tended to be more adaptable to high yielding environments, whereas GMH2 was specifically adapted to high yielding environments. However, five superior genotypes were identified (GMH146, GMH113, GMH170, GMH155 and GMH124). It was concluded that AMMI-2 is a good model to use to select for superior genotypes and best environments for genotype evaluation as this was confirmed by clustering of genotypes in a dendrogram. Observation of significant GXE, especially the cross-over type for some hybrids indicates that hybrids need to be tested in several years and locations to identify superior and stable hybrids. This would imply that hybrids produced will be capable of using resources that are available in high yielding environments and perform well.

Key words: Maize**,** Genotype X Environment Interaction, AMMI, Cultivar superiority, Stability

3.1 Introduction

The hybrid performance is determined by the genotype and environment main effects and between genotype x environment (GXE) interaction effect (Martin, 2004). Thus, a breeding program should take into account the interaction of the genotype with the environment (Abdurahman, 2009). Babić et al. (2011) reported that plant breeders neglect the GXE interaction and select varieties for advancement on the basis of the average values of the additive main effects. Unfortunately selection for broad adaptation is made complicated by GXE (Martin, 2004; Abdurahman, 2009; Babić et al., 2011). Consideration of GXE is crucial in sub-Saharan Africa because of fluctuation in environmental conditions, drought, low soil fertility, non-uniform management practices and prevalence of diseases and pests which all contribute to large GXE (Martin, 2004). Plant breeders solve this problem by planting genotypes over a wide range of environments and years to ensure that the selected genotypes have a high and stable performance over a wide range of environments (Martin, 2004). Crossa (1990) cited three of the objectives for multi-environment trials in maize as: (a) to accurately estimate and predict yield, (b) to determine yield stability and pattern of response of genotypes across environments, (c) to identify best hybrids for advancement in the breeding program. This is because a good hybrid is the one that performs well across environments. Thus, selecting the best hybrid based on the performance from one environment is not enough as no environment remains constant over the years.

Genotypes are evaluated in multi-location and seasonal environments based on their phenotypic value, thereafter, selection of experimental hybrid genotypes is made (Ilker, 2011). The relationship between phenotypic and genotypic values is impaired by the large GXE interaction variation (Ilker, 2011), because a genotype can be high yielding in one environment and low yielding in another. Kandus et al. (2010) and Ilker (2011) reported that it is difficult to select superior genotypes in plant breeding due GXE complications. The response of genotypes are not parallel in all the environments (Crossa, 1990) implying that the ranks can be reversed. Martin (2004) pointed out that relative rankings of hybrids usually do not remain the same but change in different environments. This is attributable to the concept of crossing over of

genotypes, which is described as a change in a genotypes rank from one environment to another (Crossa, 1990; Nagabushan, 2008; Abdurahman, 2009). According to Crossa (1990) with crossing over type, differences among genotypes vary in direction among environments, whereas with non-crossing over GXE genotypes differences only changes in magnitudes but not in direction. Cross over GXE type delays breeding progress because different sets of hybrids are selected in different environments (Abdurahman, 2009) leaving breeders with headache regarding what to advance in the program.

Plant breeders have to develop the strategies to either breed for broad or specific adaption of genotypes. Genotypes can be classified by their behaviour as either stable or adapted to a particular environment (Kandus et al., 2010). A stable hybrid, for example, would be capable of using resources that are available in high yielding environments and perform above average in all other environments (Nagabushan, 2008; Kandus et al., 2010). On the other hand, adaptability refers to the capacity of genotypes to give high yield under specific conditions. However, a broadly adapted genotype is more superior to a specifically adapted genotype, because of fluctuation in environmental conditions in regions from year to year.

Given the foregoing, it is imperative to quantify the level of G X E interaction and its underlying causes. Kandus et al. (2010) suggests that GXE can be analysed using combined ANOVA, stability analysis and multivariate methods. The combined ANOVA is the most commonly used method (Kandus et al., 2010). Nonetheless, combined ANOVA has limitations in that, it assumes homogeneity of variance among environments required to determine genotype differences (Kandus et al., 2010). Even though this analysis manage to determine the variance due to genotype, environment and the GXE interaction, it does not explore the response of the genotypes in the non-additive term (Kandus et al., 2010). Hence the more effective approaches such as the additive main effect and multiplicative interaction (AMMI) models are suggested to study GXE in hybrids.

The AMMI model encompasses several sources such as genotype main effect, environment main effect and the interaction with 0-F interaction's Principal component analysis (PCA) axes (IPCA) and can thus be used to predict GXE (Crossa, 1990; Babić et al., 2011). Crossa (1990) reported that the AMMI model is used for, model diagnosis, to clarify GXE and to improve accuracy of yield estimates. Additionally, Babić et al. (2011) reported that the greatest benefit of AMMI is the better understanding of genotypes, environments and GXE. This basically helps in allocating genotypes to environments they are adapted to and in identifying the best environment for evaluation of genotypes. AMMI models can range from AMMI(1), AMMI(2) to AMMI(n), depending on the number of principal components used to study the interaction (Crossa et al., 1991; Kandus et al., 2010).

On the other hand, cluster analysis seeks to find natural grouping of the hybrids (Abdurahman, 2009). Cluster analysis is performed to study the patterns of groupings of genotypes and environments. Multivariate analysis is the major tool used in estimating genetic distances as it allows for the possibility of gathering many variables into one analysis (Bertan et al., 2007). The most used statistics to estimate genetic distance are Mahalanobis (D^2) and the Euclidean distance (Darbeshwar, 2000; Bertan et al., 2007). The data is then presented in a symmetrical matrix once the distance estimate between each genotype pair is obtained and then analysed by the use of a clustering/plotting procedure (Bertan et al., 2007). Cultivar superiority and rank methods are effective and simple methods that can be used to quantify genotypic stability. The difference between these methods is that one is based on both productivity and stability and the other is based on stability only. Hence, Lin and Binns (1988) defined a superior cultivar as one with a performance near the maximum in various environments. The performance of the cultivar is measured using *Pi* index, and the lower this value is the more superior the genotype is. Similarly, the smaller the mean rank value is the more stable the genotype is.

Therefore, the objectives of the study were as follows:

i) to assess the level of genotype X environment interaction
ii) to assess cultivar superiority of the new maize germplasm lines

The information would be crucial in the selection of appropriate lines for advancement in the breeding program.

3.2 Materials and methods

3.2.1 Germplasm

Single cross hybrids were developed by crossing of experimental 116 inbred lines (inbreds) with two different tropical maize testers namely PA-1 and P1. These are late maturing tropical testers with proven discrimination capacity under stress and non-stress production conditions. A set of control hybrids (commercial) was added to the population. This resulted in 190 hybrids which were evaluated across environments. Different local check hybrids were used in each environment, while SC701 was the standard check variety in all environments.

3.2.2 Experimental environments

The hybrids were planted over five environments which were constituted by location X season combination, two seasons and three locations (Table 3.1).

Table 3.1: The environmental conditions of the locations

Location	Season	Environment codes	Latitude	Longitude	Altitude m.a.s.l	Average season rainfall (mm)	Mean temperature (^{o}C)
Ukulinga	2011/12	12UKL	-29.66S	30.40E	808	75.66	18.86
Cedara	2009/10	10CED	-29.54S	30.26E	1066	79.34	24.20
Dundee	2011/12	12DUN	-28.13S	30.31E	1217	32.41	17.03
Makhathini	2009/10	10MAK	-27.39S	32.10E	77	50.49	17.70
Dundee	2009/10	10DUN	-28.13S	30.31E	1217	*	*

*Data not available for Dundee in 2010, Mean rainfall and temperature during October to June

3.2.3 Experimental design and management

The hybrids at each environment were evaluated in an alpha lattice design, 19 blocks X 10 hybrids each (10Makhathini, 10Dundee, 10Cedara) and 15 X 10 (12Dundee, 12Ukulinga), with two replications. In all five environments, the net plot length was 4 m, the distance between the stations was 0.3 m, and the distance between the rows was 0.9 m. After thorough land preparation, sowing was done by hand dibbing of seeds with two seeds per hill. Seedlings were thinned out three weeks after emergence to maintain single seedling per hill. A total of 250 Kg/ha (NPK) 2:3:4 (55 Kg/ha of N, 83 Kg/ha of P and 111 Kg/ha of K) was applied as basal fertilizer before planting, and 250 kg/ha LAN (28% of N) was applied as a top dressing four weeks after crop emergence. Weeds were controlled by chemicals such as Basagran (nutsedge), Gramoxone (all weeds) and Troopers (broadleaf weeds including morning glory). The trials were rain-fed in all the environments. Harvesting was done by hand after the physiological maturity stage. Grain yield was measured as grain mass per plot adjusted to 12.5 % grain moisture and converted to tones per hectare.

3.2.5 Data analysis

The data obtained was subjected to REML (Restricted Maximum Likelihood) tool in GenStat 14th edition to obtain ANOVA for each environment and across environments. Experimental hybrid data was analyzed as an incomplete block design. The following linear model was used:

Within environments model for data

$Y_{ijk} = \mu + r_i + G_j + b(r)_{ik} + + e_{ijk};$

Where, Y_{ijk} = Yield

μ = grand mean

r_i= effect of the ith replication

G_j = effect of the jth genotype

$b(r)_{lk}$ = effect of the lth block in k^{th} replication

e_{ijk} = random error effects

Across environments model for data

$Y_{ijk} = \mu + r_i + G_j + E_k + r(E)_{ik} + b(r*E)_{ik} + G_jE_k + e_{ijk};$

Where, Y_{ijk}= Yield

μ = grand mean

r_i= effect of the ith replication

G_j = effect of the jth genotype

E_k = effect of the kth environment

$r(E)_{ik}$ = effect of the ith replication in k^{th} environment

$b\ (r*E)_{ik}$ = effect of the blocks in the ith replication and k^{th} environment

G_jE_k = interaction effect of the jth genotype and k^{th} environment

e_{ijk} = random experimental error

The means per environment and across environment for each hybrid were predicted by GenStat and were separated using the least significant at difference (LSD) at $P < 0.05$ level. The minimum and maximum values were used to indicate the data range.

The Additive Main effects and Multiplicative Interaction model (AMMI)

AMMI combines analysis of variance (ANOVA) into a single model with additive and multiplicative parameters. It was used to determine hybrids stability in the current study.
The AMMI model equation is (Crossa, 1990):

$$Yij = \mu + G_i + E_j + \sum_{k=1}^{n} (\lambda_k \alpha_{ik} \gamma_{jk}) + e_{ij}$$

Where, Y_{ij} = is the yield of the ith genotype in the jth environment

μ = is the grand mean

G_i = genotype deviations from the grand mean

E_j = environment deviations from the grand mean

λ_k = is the Eigen value of the PCA analysis axis k

α_{ik} = genotype principal component scores for axis k

γ_{jk} = environment principal component scores for axis k

n = number of principal components retained in the model

e_{ij} = random experimental error

Cultivar performance measure (*Pi*) was performed in GenStat as follows (Lin and Binns, 1988)

$$Pi = \sum_{j=1}^{n}\left(\frac{Xij - Mj}{n}\right)^2$$

Where, Pi = Mean square between the cultivar's yield and the overall yield for each location

X_{ij} = yield of ith genotype grown in j^{th} location.

M_j = Maximum yield response among all cultivars in j^{th} location.

n = number of locations.

Rank mean was performed in GenStat as follows (Huhn, 1979 as cited by Aremu et al., 2007):

$$S^3 = \sum_j\left(\frac{rij - ri}{ri}\right)^2$$

Where, S^3 = Non-parametric statistic

r_{ij} = rank of ith genotype in j^{th} environment

r_i = mean of ranks over all environment for i^{th} genotype

Cluster analysis

Cluster analysis was performed to study the patterns of groupings of genotypes and environments. The dendrograms were generated from GenStat multivariate hierarchical clustering (furthest neighbour) based on Euclidean distances using AMMI adjusted means.

3. 3. Results

3.3.1 Characterization of the test environments

The seasonal distribution of rainfall of the environments is presented in Figure 3.1 to Figure 3.4. Cedara (2009/10) experienced the highest mean rainfall followed by Ukulinga (2011/12). In contrast, Dundee (2011/12) had the lowest rainfall. In addition, Cedara (2009/10) exhibited the highest temperature (24.20^{o}C) and Ukulinga (2011/12) had the lowest mean temperature (18.18^{o}C) (Table 3.1).

The season started with below average rainfall but the situation improved significantly in January to April when surplus rainfall was received which averted drought at the critical reproductive growth stage and grain filling (Figure 3.1).

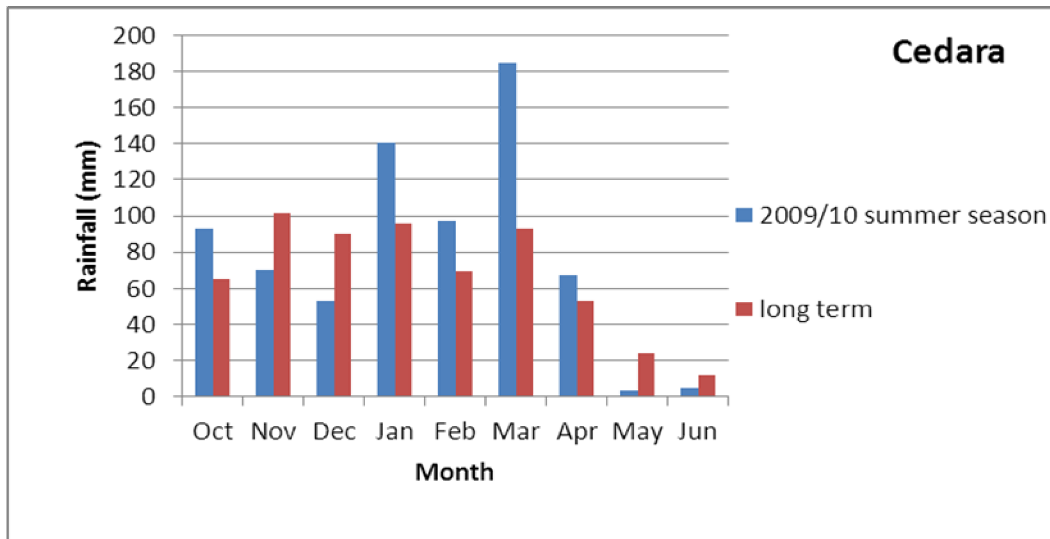


Figure 3.1: Long term (4 years back) and 2009/10 summer season mean rainfall for Cedara (Agricultural Research Council-ISCW Agromet Potchefstroom, 2012)

Rainfall was below average for all the months except March and April, basically rainfall was below the normal distribution of rainfall during flowering (Figure 3.2).

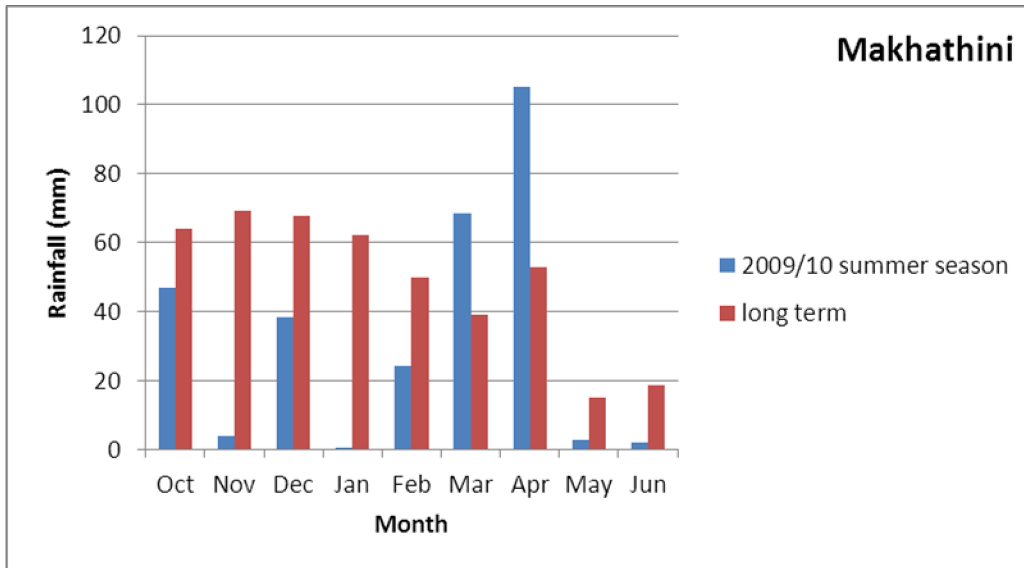


Figure 3.2: Long term (5 years back) and 2009/10 summer season mean rainfall for Makhathini (Agricultural Research Council-ISCW Agromet Potchefstroom, 2012)

There was drought in January and generally the rainfall was below average for all the other months except February (Figure 3.3).

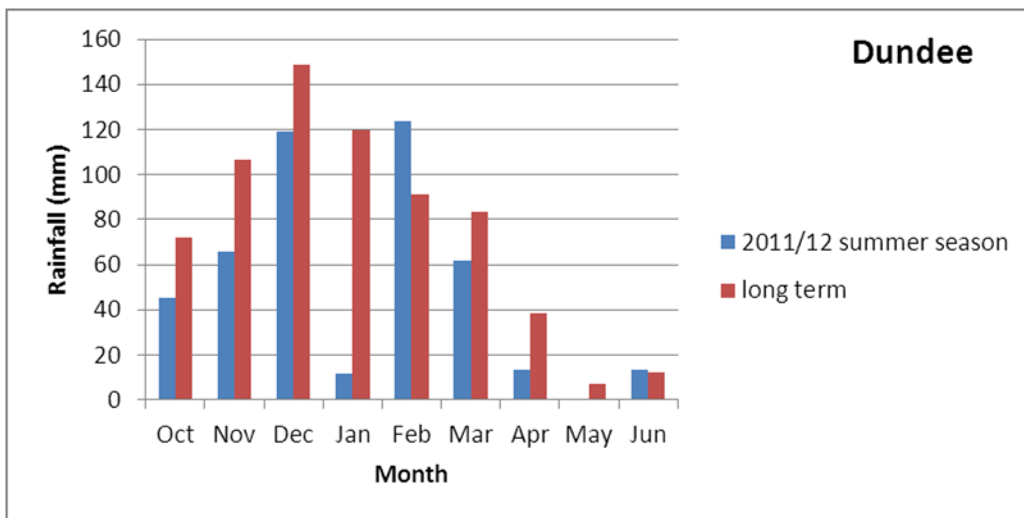


Figure 3.3: Long term (12 years back) and 2011/12 summer season mean rainfall for Dundee (Agricultural Research Council-ISCW Agromet Potchefstroom, 2012)

There was surplus rain at the beginning and near end of season; but the rainfall deficit during January and February coincided with the critical stages of silk emergence and grain filling (Figure 3.4).

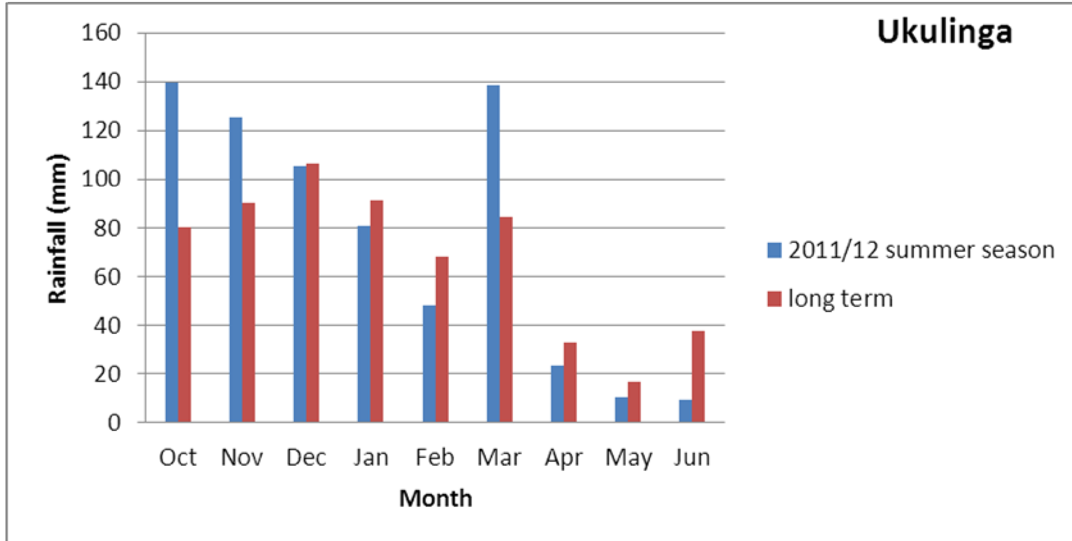


Figure 3.4: Long term (17 years back) and 2011/12 summer season mean rainfall for Ukulinga (Agricultural Research Council-ISCW Agromet Potchefstroom, 2012)

3.3.2 Genotype x environment interaction

The genotypes and environments were highly significantly different from each other and their interaction was significant (Table 3.2).

Table 3.2: ANOVA table for REML model

Fixed term	Wald statistic	DF	Wald/DF	chi pr
Genotype	762.12	116	6.57	<0.001
Environment	1856.12	4	464.03	<0.001
Genotype X Environment	547.05	464	1.18	0.005

Mean performance of hybrids across environments

The hybrids were highly significantly (P<0.01) different from each other in all the five environments (Table 3.3). The mean performance data for the selected hybrids is presented in Table 3.3. The highest yielding environment was 10Cedara followed by 10Dundee and the lowest was 12Ukulinga. Genotype 10 (GMH113) was ranked number 2 across environments and genotype 84 (GMH42) was ranked least across environments. In addition, genotype 10 (GMH113) was ranked in the top 14 in all environments, highest (ranked 1) in 10Cedara and lowest (ranked 14) in 12Dundee and it had the lowest mean rank of 18. Genotype 84 (GMH 42) was ranked 117 in two environments (10 Dundee and 10Makhathini) and across environments and ranked 84 at 12kulinga and had the highest mean rank of 354. Over 25 hybrids in this study performed better than the control (SC701). The control (SC701) was ranked 42 at 10Cedara and 10Dundee and ranked 117 at 12Ukulinga. It performed better at 12Dundee as it was ranked 11

Table 3.3: Means grain yield (t ha^{-1}) and trial data of hybrids evaluated across and within five environments (hybrids ranked by mean rank)

Genotype		Across environments		10Cedara		10Dundee		10Makhathini		12Dundee		12Ukulinga		Overall rank
Name	Code	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	# average rank
							Top 10							
GMH113	10	5.45	2	8.14	1	8.06	5	4.69	8	4.32	14	2.55	10	18
GMH146	36	5.66	1	8.13	2	7.69	8	4.04	19	5.61	3	1.97	36	30
GMH124	19	5.25	5	7.66	4	7.19	15	4.56	11	3.63	33	2.28	19	38
GMH177	61	4.95	8	7.63	5	6.79	24	5.22	4	3.45	39	1.49	61	43
GMH170	56	5.35	3	6.81	16	8.80	3	3.98	22	4.58	9	1.57	56	43
GMH115	11	4.82	11	6.25	29	8.03	6	5.07	5	3.37	40	2.53	11	50
GMH130	24	5.01	6	8.09	3	7.13	17	3.88	24	3.97	24	2.12	24	50
GMH155	45	5.27	4	5.98	38	9.09	2	4.83	7	4.15	22	1.75	45	53
GMH167	53	4.99	7	7.17	8	6.53	33	4.57	10	4.40	13	1.58	53	54
GMH139	29	4.89	10	6.85	12	7.71	7	3.68	29	3.79	29	2.03	29	55
							Control							
SC701	117	4.05	45	5.91	42	6.06	42	1.717	100	4.58	11	0.08	117	187
							Bottom 5							
GMH27	73	2.29	111	4.14	105	3.65	100	1.88	94	1.16	108	1.22	73	326
GMH21	70	1.84	116	4.62	85	2.32	116	0.92	116	0.81	113	1.24	70	345
GMH46	87	2.13	113	4.09	106	3.17	108	1.12	112	1.47	97	0.92	87	350
GMH28	74	2.00	115	2.93	117	3.14	110	1.36	107	2.42	73	1.15	74	352
GMH42	84	1.83	117	4.51	92	2.32	117	0.92	117	1.12	110	0.97	84	354
							Trial statistics							
	P-value	<0.001		<0.001		<0.001		<0.001		<0.001		0.002		

SED	0.51	1.07	1.08	0.89	1.07	0.80
Mean	3.64	5.51	5.39	2.88	2.85	1.49
Min	1.83	2.93	2.32	0.92	0.64	0.08
Max	5.66	8.14	9.33	5.88	6.13	3.83

Average rank = arithmetic mean of analytical rank values across the five environments

The main factors, genotype and environment and their interaction were highly significant (p<0.01) for hybrid yield. Additionally, IPCA1 and IPCA2 were also highly significant (p<0.01) whereas, IPCA3, IPCA4 and IPCA5 were not significant (p>0.05); hence, AMMI-2 model was adopted in this study. IPCA 1 contributed 30.9% and IPCA2 contributed 29.5% to the GXE interaction, while the contribution of IPCA3, IPCA4 and IPCA5 were less than 20% (Table 3.4).

Table 3.4: ANOVA table for AMMI model

Source	DF	Sum of squares	MS	F	# F probability	% contributed
Total	1169	5269	4.51	*	*	
Treatments	584	4532	7.76	7.93	0.00000	
Genotypes	116	946	8.16	8.34	0.00000	
Environments	4	2899	724.85	19.99	0.00000	
Blocks	5	181	36.26	37.07	0.00000	
Interactions	464	687	1.48	1.51	0.00000	
IPCA1	119	269	2.26	2.31	0.00000	39.20
IPCA2	117	203	1.73	1.77	0.00001	29.50
IPCA3	115	125	1.09	1.11	0.22459	18.20
IPCA4	113	90	0.80	0.82	0.90679	13.10
IPCA5	111	0	0.00	0.00	1.00000	0.00
Residuals	-111	0	0.00	0.00	*	
Error	568	556	0.98	*	*	

MS=Mean square, IPCA= interaction principal component axis, # Data highly significant at p<0.01 and not significant at p>0.05.

3.3.3 Environment main effects

Ranking correlations between environments

The spearman ranking coefficient (r_s) correlations of hybrids between environments ranged from 0.29 to 0.59 between environments and all the rankings were positive and highly significant (P<0.01). The ranking correlation was highest between 10Dundee and

10Makhathini and the lowest between 12Dundee and 10Cedara. Between 10Cedara and 10Dundee it was 0.59, 10Cedara and 10Makhathini was 0.51, 10Cedara and 12Ukulinga was 0.47, 10Dundee and 12Dundee was 0.47, 10Dundee and 12Ukulinga was 0.52, 10Makhathini and 12Dundee 0.51, 10Makhathini and 12Ukulinga 0, 52 and lastly it was 0.49 12Dundee and 12Ukulinga.

The AMMI biplots

The AMMI biplots provide a visual expression of the relationships between the first interaction principal component axis (IPCA1) and means of genotypes and environments (Banik et al., 2010). Environments found on quadrant B and D performed above the grand mean, whereas the environments in quadrant A and B performed below the mean. IPCA1 grouped environments 12Dundee, 12Ukulinga and 10Makhathini in quadrant A; and grouped environments 10Dundee and 10Cedara in quadrant D. The IPCA2 grouped environment 12Ukulinga and 10Makhathini in quadrant C and environment 12Dundee in quadrant A, 10Dundee in quadrant B and 10Cedara in quadrant D (Figure 3.5).

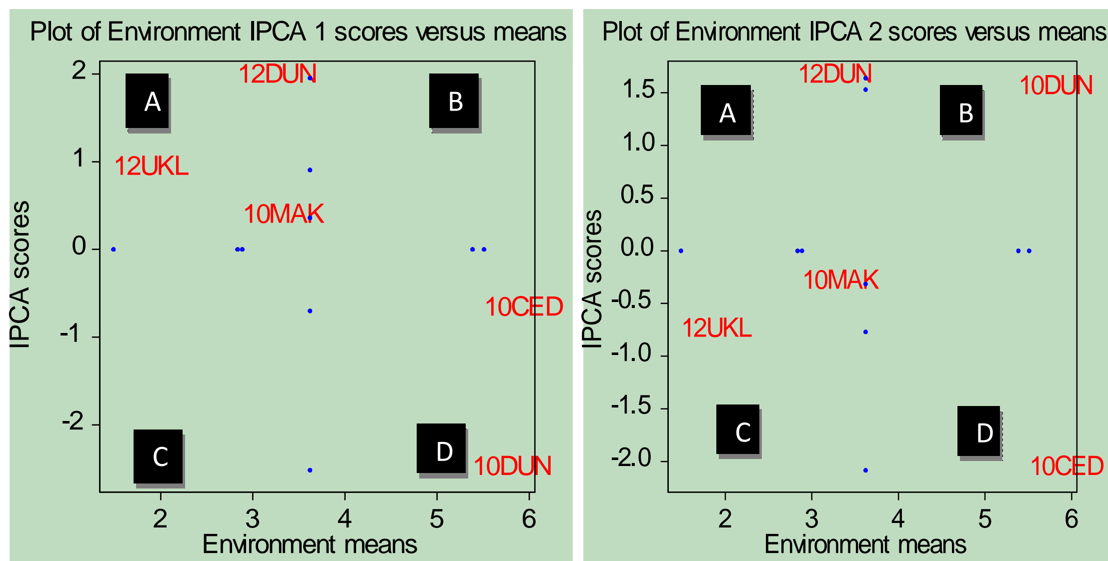


Figure 3.5: Environment means versus IPCA1 scores and IPCA2 scores

12CED=12Cedara, 10DUN=10Dundee, 10MAK= 10Makhathini, 12DUN= 12Dundee and 12UKL= 12Ukulinga, as defined in Table 3.1.

Cluster analysis of environments

There are generally two clusters (A and B) for these environments at a cut=off value 0.0, however, clustering of environments at a cut-off value of 0.9 divided cluster B in to two sub-clusters (a and b) (Figure 3.6). 10Cedara and 10Dundee were clustered in Group A and displayed 100% similarities. 10Makhathini, 12Dundee and 12Ukulinga were clustered in Group B. However, 10Makhathini and 12Dundee were more similar as they revealed 100% similarity at a cut-off of value 1.0.

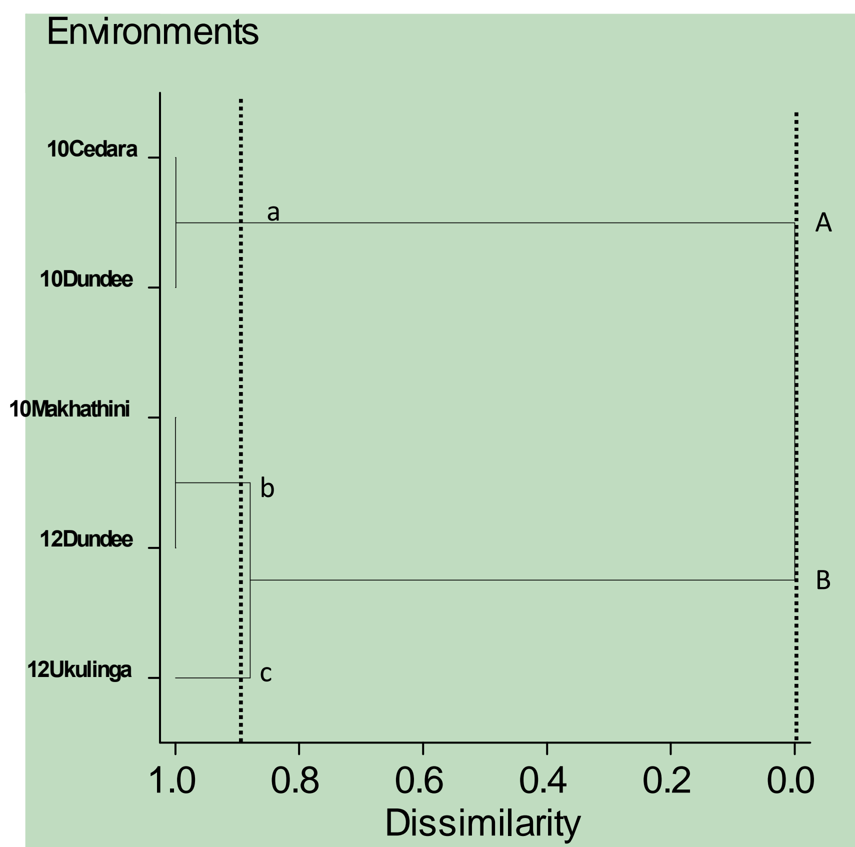


Figure 3.6: Dendrogram depicting the clustering of five environments using AMMI predicted means of grain yield for 117 hybrids

3.3.4 Genotype and environment main effect and their interaction

The IPCA scores of genotypes in the AMMI model are an indication of the stability or adaptation over environments. The greater the IPCA scores are, either negative or positive the more specific adapted is a hybrid to certain environments (İlker et al., 2009).

Environments and hybrids found on quadrant B and D performed above the mean and environments and hybrids found on quadrant A and B performed below the mean. Hybrids were found in all the quadrants, some were found next to the origin (IPCA score of zero) and some were found further away from the origin (larger IPCA score). In addition some hybrids were associated with certain environments (Figure 3.7 and Figure 3.8).

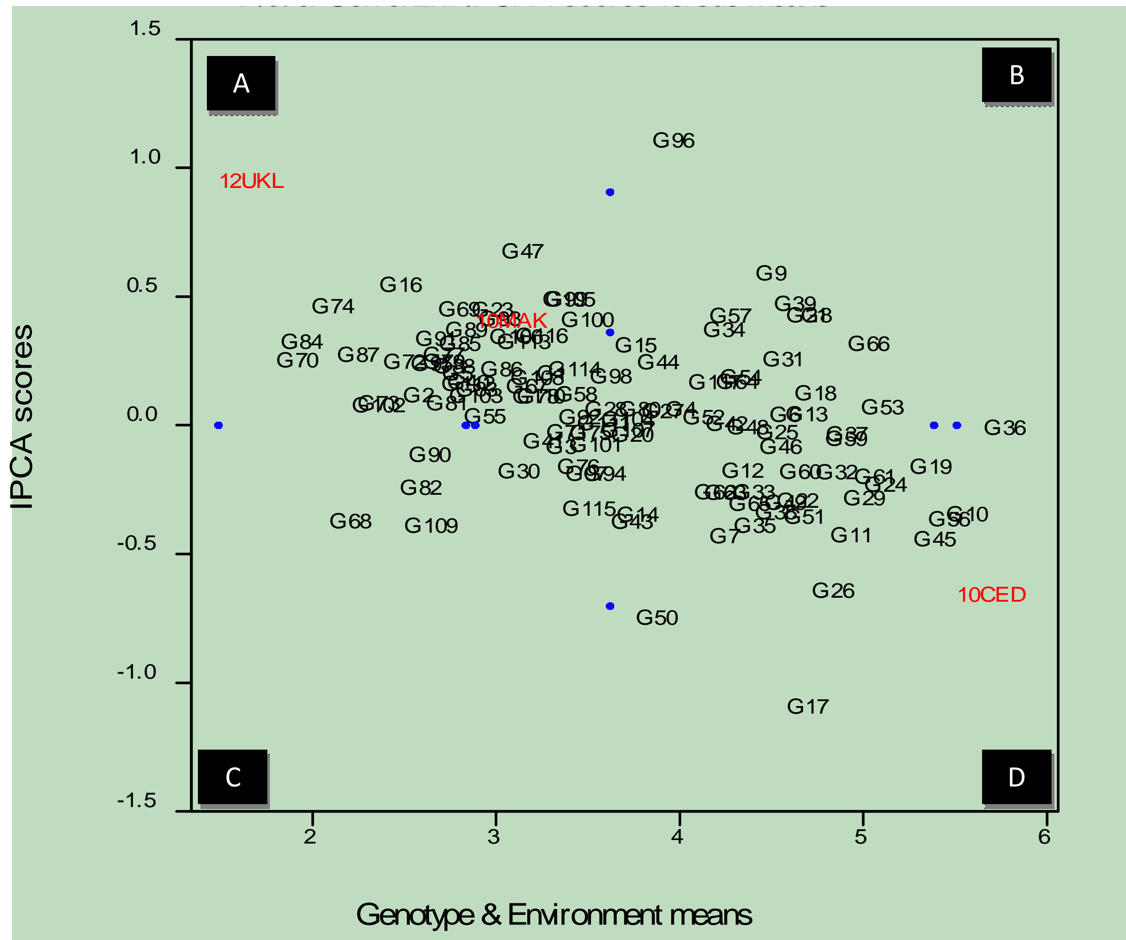


Figure 3.7: Genotype and environment means versus IPCA1 scores

(Genotype and environment codes are defined in Table 3.3 and Table 3.1 respectively)

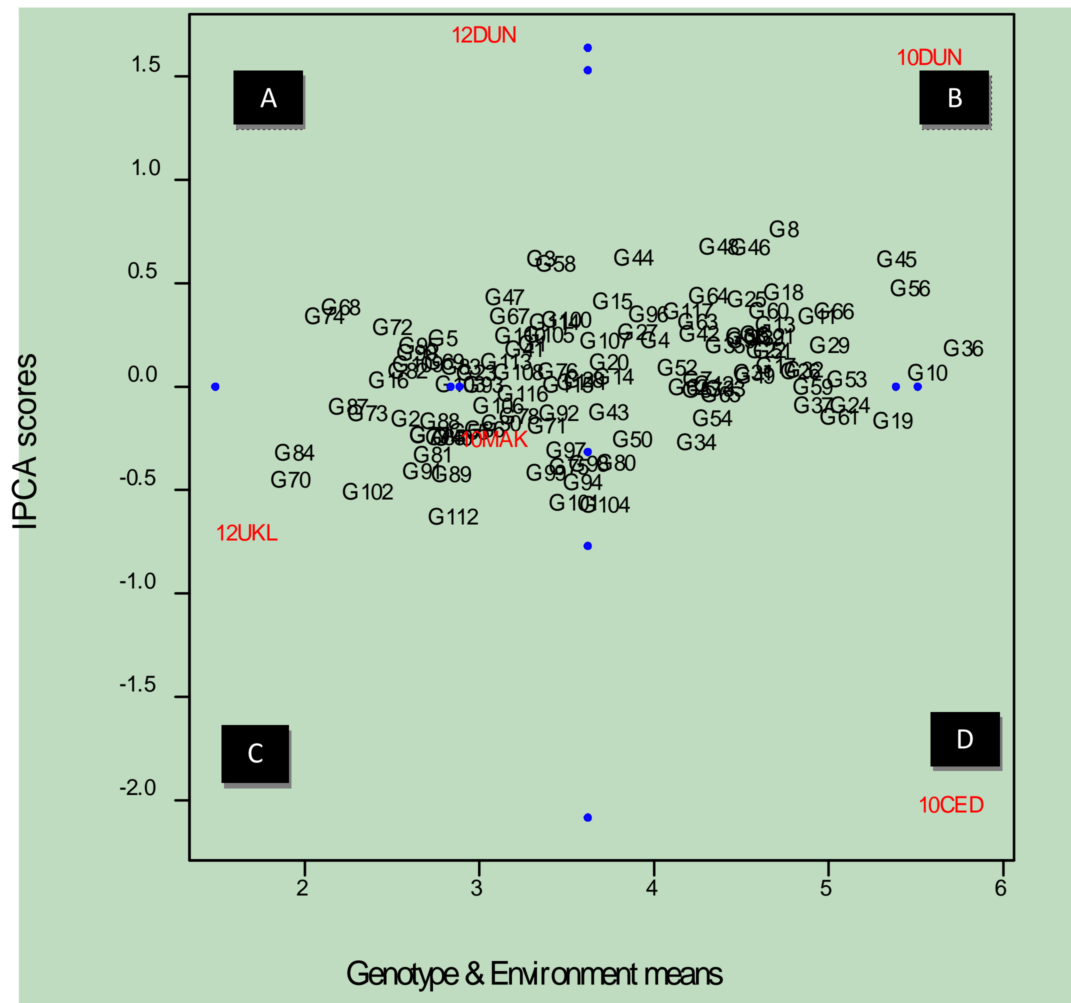


Figure 3.8: Genotype and environment means versus IPCA2 scores

(G=Genotype, 1-117= Code of genotype and environments as defined in Table 3.3 and Table 3.1 respectively)

Cluster analysis of genotypes

There are two clusters (I and II) at a cut-off value 0.0. However, clustering of the genotypes at a cut-off value of 0.8 produced four clusters (A-D). Many genotypes were in cluster C (45 genotypes) and B (40 genotypes) followed by cluster B (27 genotypes). While, only five genotypes 36 (GMH146), 45 (GMH155), 19 (GMH124), 56 (GMH170), and 10 (GMH113) were placed in cluster D (Figure 3.9).

Figure 3.9: Dendrogram depicting the clustering of 117 maize hybrids using AMMI predicted means for grain yield over 5 environments

(Genotype codes are defined in Table 3.3).

3.3.5 Cultivar superiority

The top 10 hybrids have the lowest superiority value, whereas the bottom 5 have the highest superiority value and mean rank values. Genotypes with the lowest superiority value and mean rank also had the highest yield (Table 3.5).

Table 3.5: Stability of maize hybrids using cultivar superiority method and mean rank method

Name of hybrid	Genotype code	Cultivar mean (t ha^{-1})	Cultivar superiority index	Mean rank method
		Top 15		
GMH146	36	5.66	0.74	7.20
GMH113	10	5.45	0.95	11.30
GMH170	56	5.35	0.96	11.50
GMH155	45	5.27	1.20	17.00
GMH124	19	5.25	1.32	13.20
GMH167	53	4.99	1.59	16.80
GMH139	29	4.89	1.66	18.10
GMH130	24	5.01	1.70	20.80
GMH177	61	4.95	1.90	23.90
GMH174	59	4.79	1.91	20.70
GMH181	66	4.91	1.94	25.40
GMH115	11	4.82	1.95	29.20
GMH147	37	4.80	2.05	23.40
GMH142	32	4.74	2.11	28.30
GMH121	18	4.63	2.18	28.00
		Control		
SC701	117	4.05	3.89	45.90
		Bottom 5		
GMH2	68	2.09	10.76	102.40
GMH46	87	2.13	10.78	103.00
GMH28	74	2.00	11.34	104.20
GMH42	84	1.83	12.48	109.40
GMH21	70	1.94	12.65	107.70

3.3.6 The AMMI model's best four hybrid selections

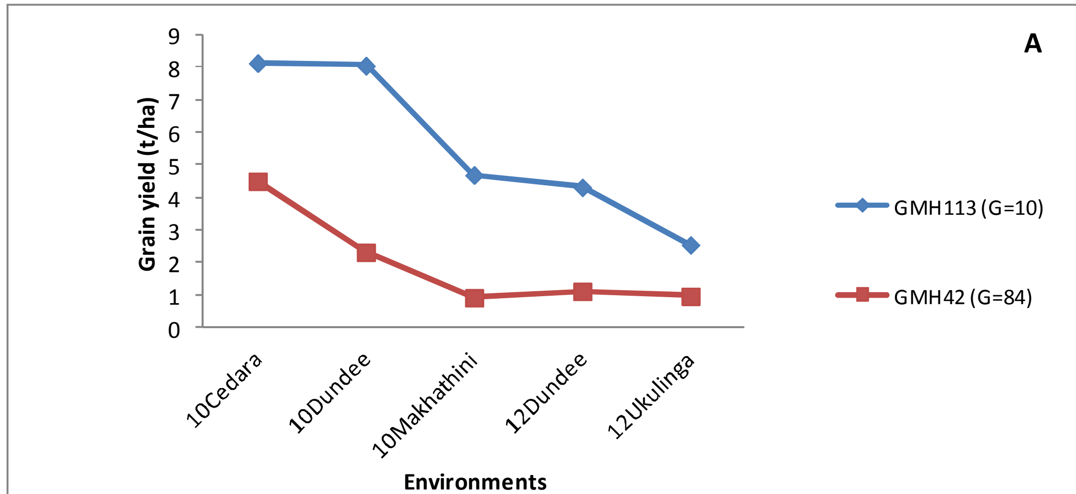
The AMMI model revealed that 10Cedara was the highest yielding environment and 12Ukulinga was the lowest yielding environment. The hybrid GMH146 was ranked number 2 at 12Dundee and 10Cedara, GMH112 was ranked 3 at 12Ukulinga and 10Makhathini, GMH181 was ranked 2 at 10Makhathini and 4 at 12Dundee and GMH124 was ranked 2 at 12Ukulinga and 4 at 10Cedara. The other genotypes are ranked in the top 4 once per environment (Table 3.6).

Table 3.6: The AMMI model's best four hybrid selections for mean grain yield in relation to the environments evaluated

Environment	Mean Yield (t ha^{-1})	IPCA Score	Hybrid Rank			
			1	2	3	4
12Dundee	2.835	1.954	GMH111	GMH146	GMH171	GMH181
12Ukulinga	1.488	0.906	GMH66	GMH124	GMH112	GMH141
10Makhathini	2.886	0.360	GMH126	GMH181	GMH112	GMH177
10Cedara	5.510	-0.702	GMH113	GMH146	GMH130	GMH124
10Dundee	5.385	-2.518	GMH120	GMH155	GMH170	GMH136

3.3.7 Observations of crossing and non-crossing rank of genotypes

Genotype 10 (GMH113) performed better that 42 (GMH42) in all environments (Figure 3.10-A). Crossing over is clearly observed between Genotype 36 (GMH146) and genotype 56 (GMH170) in all environments (Figure 3.10-B).



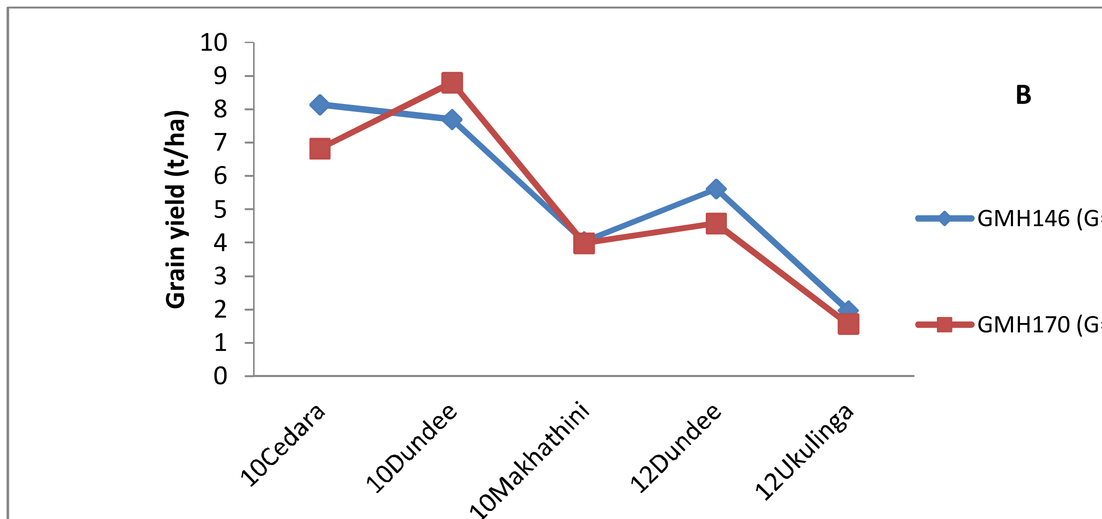


Figure 3.10: Example of non-crossing over (A) and crossing over (B) ranking of genotypes in all environments

3.4 Discussion

3.4.1 Genotype x environment interaction

The genotype main effects were significant indicating that the hybrids exhibited different yield means which provides opportunity for selection. The genotypes and environments and their interaction were highly significant in this study, this confirms that there is a need to evaluate genotypes in different locations and years to identify stable genotypes. This also indicates that in this study some genotypes were more adaptable to low input environment and some were adapted to high input environments. These results are in accord with

previous findings by Martin (2004), Nagabushan (2008) and Abdurahman (2009) and Rahman et al. (2010). Mashark et al. (2007) reported that significant GXE indicated that there were rank changes for the genotypes from location to location within a year and from year to year across locations.

In this study, IPCA1 and IPCA2 were highly significant and adequate to explain 69% of genotype X environment interaction; hence the AMMI-2 model was adopted (Table 3.4). In fact the IPC3 and IPC4 were only significant at p= 0.22 and p= 0.90, respectively, which is well above the cut-off point of P≤0.05. Whereas, IPCA5 was not significant at all (P=1.0). In addition the IPCA3, IPCA4 and IPCA5 combined explained only about 30% of the GXE. Generally, the AMMI-2 model and/or AMMI-3 model have been found to be significant to analyse GXE in many maize trials by previous researchers, such as İlker et al. (2009) and Zenebe and Hussien (2010). Therefore, the current results are consistent with previous findings in maize GXE studies that show that simple models are the most appropriate. Zenebe and Hussien (2010) reported two significant IPCA's, whereas in the study of İlker et al. (2009) AMMI analysis partitioned the sum of squares of GXE into 8 IPCA's, but only the first three were significant. Additionally, Abdurahman (2009) found that the first three IPCA's were highly significant (p<0.01) and the last two were significant at p<0.05. Therefore the findings contribute to abundant evidence that shows that simple models such as AMMI-2 are adequate to explain GXE in maize hybrid trials.

3.4.2 Environment main effect

Highly significant (p<0.01) differences were observed among genotypes in all environments for grain yield. The highest yielding environment was 10Cedara followed by 10Dundee and the lowest was 12Ukulinga, this could be due to the fact that 10Cedara experienced rainfall above the normal rainfall, mainly during flowering (Figure 3.1). On the other hand, 12Ukulinga, though it had high mean rainfall for the season, its distribution was not favourable because drought occurred at the critical stage of flowering. In general, season 2010 yielded more than season 2012, the observation demonstrate that the seasonal variation which occurs in tropical and sub-tropical environments and impacts on maize

production. This calls for introduction of genotypes with high stability to safe guard yield at the household level. These results are confirmed from IPCA1 AMMI plots as the environments 10Makhathini, 12Dundee and 12Ukulinga are grouped in quadrant A, supporting low yield in this environments implying that the two represent unfavourable environments. 10Dundee and 10Cedara were grouped in quadrant D, as they were the highest yielding environments. In addition, cluster analysis of environments confirmed the results from AMMI IPCA1 plots as it produced two clusters for these environments (Figure 3.6), where it grouped 10Cedara and 10Dundee in Group A displaying 100% similarities. Whereas, 10Makhathini, 12Dundee and 12Ukulinga were clustered in group B, indicating that these environments would discriminate genotypes similarly.

However, IPCA2 AMMI plot was able to separate all the environments to different quadrants confirming that these environments are different from each other. 12Ukulinga and 10Makhathini were put very close to each other, this could be because they both have low altitudes; hence they had similar interaction with hybrids. 10Makhathini was a more stable environment as it was found close to the origin, meaning it is not a good discriminating environment of genotypes. 10Dundee and 12Dundee were grouped in two adjacent quadrants B and A, respectively, basically, the difference between these environments is season and the location is the same, supporting that Dundee is either a high or low yielding environment depending on the season. It represents the variable environments in SSA. Therefore, Dundee is not a stable environment and hence specifically adapted genotypes cannot be recommended to Dundee area because its yield depends on the season. This therefore is complicated for breeders who want to breed for genotypes adapted to a specific location. 10Cedara was put in quadrant D as it is generally a different high yielding environment. The yield limiting factor of genotypes in other environment could mainly be attributed to rainfall and altitude. Similar observations were reported by Beyene et al. (2011) who also suggested that differential response of the genotypes across environments was due to altitude, temperature and rainfall differences. High yielding environments ranked genotypes similarly and the same was observed for the low yielding environments.

3.4.3 Genotype performance

The GXE analysis reveals the different types of stability with implication for breeding. Genotype 10 (GMH113) followed by genotype 36 (GMH146) was well ranked across environments. It yielded 8.14 t ha^{-1} (ranked 1) at 10Cedara, 8.06 t ha^{-1} (ranked 5) at 10Dundee, 4.69 t ha^{-1} (ranked 8) at 10Makhathini, 4.32 t ha^{-1} (ranked 14) at 12Dundee and 4.32t ha^{-1} (ranked 10) at 12Ukulinga (Table 3.3). This tells us that the Genotype 10 (GMH113) exemplified dynamic stability implying that it has the ability to respond positively to improved environmental conditions, as it was able to give high yield both under low inputs (12Ukulinga) and high inputs (10Cedara) environments. Similarly, it is deemed to have a general or wide adaptation. Therefore, this genotype can be recommended for advancement in the next season with a view to release it for production in variable environments. These results are confirmed from the AMMI IPCA2 plot as this genotype showed an IPCA score of zero which implies stability. However, AMMI IPCA1 plot showed genotype 36 (GMH146) to be the most stable genotype. In addition cultivar superiority index also displayed genotype 36 (GMH146) as the most stable genotype followed by genotype 10 (GMH113) (Figure 3.7 and Figure 3.8 and Table 3.5). The discrepancy between IPCA1 and IPCA2 outcome could be attributed to the fact that they are based on different factors for GXE. However, IPCA's are generally random and therefore difficult to explain.

Conversely, genotype 74 (GMH28) displayed a static type of stability meaning that it maintains almost constant yield across environments. This is confirmed from the results as genotype 74 (GMH28) yielded 2.93t ha^{-1} at 10Cedara, 3.14 t ha^{-1} at 10 Dundee, 1.36 t ha^{-1} at 10Makhathini, 2.42 t ha^{-1} at 12Dundee and 1.15t ha^{-1} at 12Ukulinga (Table 3.3) which is consistently below the mean. Hence, it is a non-desired genotype, and cannot be recommended to any environment; because it does not respond to the environments in management. Furthermore, farmers who grow this hybrid would incur some yield penalty in seasons where rainfall is above average. This particular hybrid performed below the mean in all environments and for this reason it will be discarded from the program. Both the AMMI IPCA1 and IPCA2 proved that this hybrid possessed static stability as it was found in quadrant A (low yielding) with an IPCA score less than 0.5. Additionally it was found on the bottom 5 in cultivar superiority results (Figure 3.7 and Figure 3.8 and Table 3.5). This hybrid

represents many genotypes which will be discarded for poor dynamic stability and low productivity.

Moreover, genotype 68 (GMH2) yielded 3.06 t ha-^{1} at 10Cedara, 5.55 t ha-^{1} at 10 Dundee, 0.93 t ha^{-1} at 10Makhathini, 0.604 t ha^{-1} at 12Dundee and 1.36 t ha^{-1} at 12Ukulinga. This, undoubtedly, displays that this genotype is specifically adapted to high yielding environments, hence it can only be recommended to favourable environments which represented by these test environments. Similarly, Choukan (2011) reported that for specific adaptation, the ideal genotype should have high mean grain yield and respond well to a particular environment. Since all the top 10 genotypes performed better than the control (SC701) in the current study, this confirms the presence of potential hybrids to be released to the farmers, and represents a show of breeding progress that has been realised. In contrast, this genotype (SC701) was found on the same quadrant as 12Dundee and adjacent to 10Dundee in the IPCA-2 plots, revealing that it was more adaptable to the Dundee location, and would give competition to the new hybrids in that environment.

The mean rank and cultivar superiority index ranked hybrids in a less similar manner. However, cultivar superiority index is more reliable as it ranks genotypes according to their performance and stability, whereas mean ranks is according to stability alone. The AMMI-2 plots showed that 12Ukulinga and 10Cedara interacted more with the genotypes as they are found further away from the genotypes. Therefore, they are the most discriminating environments of genotypes (Abdurahman, 2009) and contributed more to GXE interaction (Broccoli and Burak, 2004). Whereas, environment 10Makhathini was found within genotypes indicating that it had a little interaction with the hybrids. Cluster analysis grouped the five stable hybrids in cluster D while, hybrids in cluster C had moderately high stability (Figure 3.6). On the contrary, hybrids in cluster B had the lowest stability, whilst hybrids in cluster A had moderately low stability. Similar results were found in the AMMI-1 and AMMI-2 biplots and from the cultivar superiority index (Figure 3.7 and Figure 3.8 and Table 3.5). This indicates that genotypes clustered on the same group may have the same gene frequency conferring grain yield stability and can belong possibly to the same heterotic group.

3.4.4 Crossing over ranking of genotypes

Non-crossing over ranking of hybrids was observed, for example, genotype 10 (GMH113) performed better than genotype 84 (GMH42) in all environments illustrating that the GXE observed was partly due to differences in magnitudes of means. This suggests that this hybrid is more stable and can thus be recommended in all environments. In contrast, crossing over was observed between genotype 36 (GMH46) and genotype 56 (GMH170). This implies that genotypes are ranked differently from one environment to the other, meaning that top 10 genotype in one environment are not necessarily the top ten genotypes in another environment. This is problematic as it delays breeding progress, and consequently genotypes with high average performance cannot be recommended in all environments. These results are in line with Ilker (2011) who reported both cross over and non-cross over GXE in maize hybrids with the former being more challenging.

3.4.5 The AMMI model's best four hybrid selections

The relationships of genotypes and environments and summary patterns were successfully obtained from the AMMI-2 model. It showed the best adapted hybrids in relation to the different environments. The AMMI model revealed that 10Cedara was the highest yielding environment and 12Ukulinga was the lowest yielding environment. Genotype 9 (GMH112) was ranked 3 at 12Ukulinga and 10Makhathini; this indicates that this hybrid is specifically adapted to low yielding environments, yet, it still gave above average yields at high yielding environments, which supports the observation of dynamic stability. In addition, genotype 66 (GMH181) was ranked 2 at 10Makhathini and 4 at 12Dundee suggesting that it is specifically adapted to moderate yielding environments. On the other hand, genotype 36 (GMH146) is ranked 2 at 12Dundee and 10Cedara, demonstrating that this genotype is adaptable to both unfavourable and favourable environments, respectively. It has a general adaptability and therefore dynamic. Furthermore, Genotype 19 (GMH124) is ranked 2 at 12Ukulinga and 4 at 10Cedara; this reveals that it encompass a general adaptation as it is adapted to both unfavourable and favourable conditions. The other hybrids are ranked once per environment, they did not show any distinctive pattern, it was either they were adapted to

unfavourable or favourable environments implying that the study returns hybrids with both specific and wide adaptation. These results show that AMMI-2 is a good model to use to select superior genotypes and best environments for genotype evaluation. Similar results were observed by Martin (2004).

3.5 Conclusion

From the study it can be concluded that,

- There was a highly significant genotype X environment interaction, particularly the crossing over interaction; this indicates that there is a need to evaluate genotypes in different locations and years. However, the non-crossing over type was also observed.
- The study divided the environments into two groups, high and low yielding environments which provide the opportunity to select the hybrids within the two groups for recommendation to high and low yielding environments which are represented by this set of test environment.
- Mean rank revealed GMH113, GMH146, GMH124, GMH177 and GMH170 hybrids to be most stable
- The AMMI-2 revealed GMH113, GMH146, GMH124, GMH167 and GMH130 hybrids to be the most stable
- The cultivar superiority displayed the following hybrids to be more stable GMH146, GMH113, GMH170, GMH155 and GMH124

Both the AMMI-2 model and Stability methods identified a similar set of hybrids, which were also ranked in the top 5. Therefore, selection of these hybrids for advancement in the program is reliable. This set of hybrids displayed immense superiority over the standard hybrids qualifying them as possible candidates for advancement.

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CHAPTER FOUR

Combining Ability and Genetic Variation among New Maize Germplasm Lines

Abstract

Combining ability data is important for maize breeders who emphasize hybridization and selection as means of producing high yielding varieties. The objective of the study was to assess combining ability, genetic parameters and correlation between grain yield and secondary traits of the new maize germplasm lines. Therefore, 190 hybrids were evaluated in five environments comprising of three sites and two seasons. The hybrids were laid out in an incomplete block design with two replications. The REML tool from GENSTAT was used to perform the line X tester analysis. Results indicate significant line and tester main effects, and line X tester interaction effects ($p<0.01$), implying that GCA which is attributable to both lines and testers, and SCA effects, respectively, are important in governing grain yield. Four lines GML68, GML58, GML86 and GML13 displayed large and significant GCA effects for yield. Additionally, five crosses with large positive SCA effects for yield were identified. It was also observed that crosses involving lines with negative GCA also gave hybrids with positive SCA effects indicating that dominance gene action also played a significant part in influencing the yield of hybrids. However, additive gene action contributed more to the inheritance of grain yield in the hybrids. In addition, heritability of grain yield was the highest in all environments ranging from 85 to 94%, whereas heritability for the secondary traits such as number of ears per plant, anthesis date, grain moisture and plant height ranged from low to high. Consistent with the literature there was a significant relationship between grain yield and most of the secondary traits. Nonetheless, in this study it can be concluded that the number of ears per plant and anthesis date could be the most reliable secondary traits to improve yield via indirect selection. However, the observation of large heritability estimates for grain yield in this set of germplasm lines supports direct selection strategy.

Key words: Maize, Line X tester, General Combining Ability, Specific Combining Ability, Heritability, Grain yield, Secondary traits

4.1 Introduction

Importance of developing maize hybrids to improve food security cannot be over emphasized. The acreage and productivity of maize was increased by hybrid cultivars over the years (Kanagarasu et al., 2010) because hybrids exhibit vigour. The combining ability of an inbred is measured by its ability to combine with other inbreds to produce superior hybrids (Bello and Olaoye, 2009). Additionally, combining ability is the relative ability of a genotype to transmit its desirable performance to its progenies. Maize breeders are interested in identifying inbred lines that would combine well and give high yields without making all possible crosses among the potential parents (Makumbi et al., 2011) which can be costly and time consuming. Sprague and Tatum (1942) defined general combining ability (GCA) as "the average performance of a genotype in hybrid combination, while specific combining ability (SCA) as those cases in which certain combinations perform relatively better or worse than would be expected on the basis of the average performance". Knowledge of GCA and SCA data and their relative contribution to hybrids is crucial for breeders to devise on appropriate strategy.

In maize breeding, combining ability data is crucial to improve hybrids (Farhan et al., 2012). This knowledge is very imperative for the selection of suitable parents for hybridization, and selection of new hybrids (Bocanski et al., 2011). The combining ability data can be used to make inferences about gene action. General combining ability is mainly influenced by additive gene effects and additive X additive interaction variance while SCA is influenced by variance due to dominance variance, and additive X additive variance, additive X dominance variance and dominance X dominance variance components of epistasis (Rojas and Sprague, 1952; Darbeshwar, 2000). The variance due to GCA is usually considered to be an indicator of the extent of additive type of gene action, whereas SCA is taken as the measure of non-additive gene effects (Kanagarasu et al., 2010). Additive gene actions are more important in determining traits in the populations whereas non-additive gene effects would be important in inbred line crosses (Kebede, 1989). Younes and Andrew (1978) reported that for most traits in previously unselected material additive gene action is more important than non-additive components. This is because additive gene action is independent of other genes and the environment.

Grain yield is a complex trait which is controlled by the interaction of many genotypic and environmental factors (Selvaraj and Nagarajan, 2011) hence breeding for it is difficult. It might sometimes be possible to practice indirect selection for grain yield using suitable secondary traits. Munyiri et al. (2010) reported that the genetic variance and heritability of grain yield decreases under stressful conditions. In contrast, the genetic variance and heritability for secondary traits increase under stressful conditions and can thus be used to improve grain yield (Bänziger and Lafitte, 1997). Edmeades et al. (1998) established that an ideal secondary trait for indirect selection must be genetically associated with grain yield under stress, highly heritable, cheap and fast to measure, and stable within a measurable period. Further, Edmeades et al. (1998) assert that a suitable secondary trait is not associated with a yield penalty under favourable conditions, should be observed at or before flowering, and a reliable estimator of yield potential before final harvest. In addition, Kashiana et al. (2010) reported that there are only a few yield components which are controlled by fewer genes, with high heritability. The relationship between yield and secondary traits can significantly improve the efficiency of breeding programmes (Mohammadi et al., 2003) if it is properly quantified.

Govindaraj et al. (2010) asserts that estimation of genetic parameters such as heritability, would be useful in developing appropriate selection strategies. This is because the heritability of a trait determines its expression in the next generation. Govindaraj et al. (2010) described heritability as a measure of possible genetic advancement under selection. It measures the transmission of characters from one generation to another (Govindaraj et al., 2010). Thus, partitioning of observed variability into its heritable and non-heritable components is necessary in breeding. Furthermore as genetic coefficient of variation, heritability and genetic advancements should be established. Govindaraj et al. (2010) reported that reliability of variance value as a selection guideline can be expressed by heritability estimates of quantitative traits such as yield. Additionally, Sečanski et al. (2004) reported that from evaluation of heterosis and, genetic variability is also very important.

Therefore, the objectives of the study were as follows:

i) to assess combining ability of maize germplasm lines

ii) to determine genetic parameters

iii) To determine the correlation between grain yield and secondary traits of the new maize germplasm lines.

The information would be used to device a suitable strategy to improve breeding efficiency and effectiveness.

4.2 Materials and methods

4.2.1 Germplasm

Single cross hybrids were developed by crossing of 40 experimental inbred lines with two different testers namely (PA-1 and P1). These are late maturing tropical testers with proven discrimination capacity under stress and non-stress production conditions. A set of control hybrids (commercial) was added to the population. Different local checks were used in each environment, while SC701 was the standard check variety in all environments. The same germplasm was used in chapter 3.

4.2.2 Experimental environments

The test environments are described in Table 3.1 (see chapter 3). The experimental design and management is described in chapter 3 (see section 3.2.3).

4.2.4 Data collection

The following traits were measured in all the environments following the standard protocols used at CIMMYT (Magorokosho et al., 2009):

I. **Plant height (PH)** was measured as the distance between the base of a plant to the insertion point of the top ear in cm. It was measured when all the plants have flowered in meters, since plants reach their maximum height at flowering.
II. **Ear height (EH)** was measured as height from ground level up to the base of the upper most cobs bearing internode in meters in cm.

III. **Ear position (EPO)** was measured as the ratio of ear height to plant height. Small values indicate low ear position and large values will indicate high ear position.

IV. **Anthesis date (AD)** was measured as the number of days after planting when 50% of the plants shed pollen.

V. **Silking date (SD)** was measured as the number of days after planting when 50% of the plants produced silks.

VI. **Anthesis-silking intervals (ASI),** the number of days after planting when 50% of the plants shed pollen (anthesis date, AD) and extrude silks (silking date, SD) were recorded and ASI calculated as ASI = SD – AD.

VII. **Root lodging (RL)** was measured as percentage of the plants per plot which have their stems inclining by more than 45°.

VIII. **Stem lodging (SL)** was measured as the percentage of plants per plot that have their stems broken below the ear.

IX. **Number of ears per plant (EPP)** was measured by counting the number of ears per plot and divided by the number of plants.

X. **Ear length (EL)** was measured from the tip of the ear to the base in cm.

XI. **Grain moisture (MOI)** was measured as a percentage of water content of grain at harvest.

XII. **Grain yield (GY)** was measured as grain mass per plot adjusted to 12.5 % grain moisture and converted to tones per hectare.

XIII. **Grain texture (TEX)** was rated on the scale from 1 (= flint) to 5 (= dent).

4.2.5 Data analysis

Analysis of line X Tester

The quantitative data for all traits was subjected to a line × tester analysis using the REML (Restricted Maximum Likelihood) tool in Genstat following a fixed model for the individual site data, using the model:

$$Y_{ijkl} = \mu + r_i + L_j + T_k + E_l + LXT_{jk} + LXE_{jl} + TXE_{kl} + LXTXE_{jkl} + e_{ijkl};$$

Where, y_{ijkl} = observed hybrid response

μ= overall trial mean

r_i = replication

T_k = effect of the Kth tester

L_j = effect of the Jth line

LXT_{jk} = effect of the interaction of jth line and k^{th} testers

LXE_{jl} = effect of the interaction of jth line and l^{th} environments

TXE_{kl} = effect of the interaction of kth tester and l^{th} environments

$LXTXE_{jkl}$ = effect of the interaction of jth line, k^{th} tester and l^{th} environments

e_{ijkl} = random experimental error

The GCA effects for each line and tester were estimated as follows (Shashidhara, 2008):

$$\text{Lines} = \frac{Xi \ldots.}{mr} + \frac{X \ldots \ldots}{mfr}$$

Where,

Xi….. = total of ith female parent over all male (m) parents and replications (r).

X… = Grand total of all the hybrids over all male parents (m), female parents (f) and replications (r).

$$\text{Testers} = \frac{Xj \ldots.}{fr} + \frac{X \ldots \ldots}{mfr}$$

Where, X_j… = Grand total of the jth male parent over all female parents (f) and replication (r).

The standard error (SE) for line and tester GCA effects were estimated as follows (Dobholkar, 1999 as cited by Makanda, 2009):

$$\mathbf{SE_{Line}} = \sqrt{\frac{MSE}{S * T}}$$

$$SE_{Tester} = \sqrt{\frac{MSE}{S * L}}$$

Where: MSE = mean square error; S = number of sites; L and T = number of lines and testers, respectively.

The t-tests were calculated to determine the significance of lines and testers as follows:

$$t_x = \frac{GCA_X}{SE_X}$$

Where: t_X = t-statistic of either line or tester

GCA_X = general combining ability for either line or tester

SE_X = standard error of line or tester;

The SCA effects for each line and tester were estimated as follows (Shashidhara, 2008)

$$SCA = \frac{Xij}{r} - \frac{Xi \ldots.}{mr} - \frac{Xj}{fr} + \frac{X \ldots \ldots}{mfr}$$

Where, X_{ij} = ji^{th} combination total over all replications (r).

The standard error (SE) for line by tester SCA effects estimated as follows (Dobholkar, 1999 as cited by Makanda, 2009):

$$SE = \sqrt{\frac{MSE}{S}}$$

The t-tests were calculated to determine the significance of line by tester interaction as follows:

$$t_x = \frac{SCA_X}{SE_X}$$

Association analysis

The correlation coefficients were calculated using GenStat 14th edition.

Estimation of genetic parameters

Genetic parameters were estimated for different traits on maize genotypes as follows:

Heritability (H^2)

Heritability in a broad sense was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage (Darbeshwar, 2000).

$$H^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma e^2/rs} \text{ X } 100 \text{ (Across environments)}$$

$$H^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e/r} \text{ X } 100 \text{ (Within environments)}$$

Where, $\sigma^2 g$ = Genotypic variance, $\sigma^2 e$ = environmental variance, r= replication and s = site)

Genetic advance

The extent of genetic advance to be expected by selecting five per cent of the superior progeny was calculated by using the following formula given by Robinson et al. (1949).

$$GA = i\ \sigma_p\ h^2$$

Where,

i = efficacy of selection which is 2.06 at 5% selection intensity

σ_p = phenotypic standard deviation

H^2 = heritability in broad sense

Genetic advance as per cent of mean

$$\text{GA as per cent of mean} = \frac{GA}{X} \text{x } 100$$

Where,

GA = genetic advance

X = general mean of character

Genotypic and phenotypic coefficient of variation

The genotypic and phenotypic coefficient of variation was computed according to Burton and Devane (1953) as cited by Darbeshwar (2000) and expressed as percentage.

Genotypic coefficient of variation: $GCV = \frac{\sqrt{\sigma 2 g}}{x} \text{X } 100$

Phenotypic coefficient of variation: $PCV = \frac{\sqrt{\sigma 2p}}{X} X\ 100$

Where,

$\sigma^2 g$ = Genotypic variation, $\sigma^2 p$ = Phenotypic variation and X =general mean of the character.

4.3 Results

4.3.1 Combining ability for yield based on 40 lines x 2 testers

Only testcrosses with adequate seed were evaluated in all trials; hence combining ability study was based on 40 lines X two testers. The lines and testers main effects and line X tester interaction effects were highly significant ($p<0.01$) for grain yield. The interaction between the environment X line, environment X testers and environment X line X testers was significant ($p<0.05$) (Table 4.1).

Table 4.1: REML model for grain yield across environments

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr	% contribution to hybrids
Line	141.16	39	3.62	<0.001	15.55
Tester	593.20	1	593.20	<0.001	65.37
Site	2554.27	4	638.57	<0.001	
Line X Tester	173.06	39	4.44	<0.001	19.07
Line X Site	259.57	156	1.66	<0.001	
Tester X Site	88.49	4	22.12	<0.001	
Line X Tester X Site	280.57	156	1.80	<0.001	

4.3.2 Combining ability effects

The GCA effects of the lines are shown in Figure 4.1. In the current study, positive GCA effects are desired because they reflect contribution of the line in its hybrid. Nineteen lines exhibited a positive GCA effects and 21 lines displayed a negative GCA effects. GML68 and GML58 showed a highly significant ($p<0.01$) and positive GCA effects. Additionally, GML86 and GML13 presented highest positive and significant ($p<0.05$) GCA effects. In contrast, GML43, GML66 and GML27 demonstrated the highest negative and highly significant

(p<0.01) GCA effects, however, GML3 exhibited a large negative and significant (p<0.05) GCA effect. Tester P1 had a significant (p<0.05) and positive GCA effect of 0.746 and tester PA-1 had a significant (p<0.05) and negative GCA effect of 0.747.

The SCA effects for the lines crossed with PA-1 are shown in Figure 4.2. Twenty one lines displayed a positive SCA effect with PA-1, whereas, 19 lines exhibited a negative SCA effects when crossed with PA-1. GML18 and GML3 had the highest positive and significant (p<0.01) SCA effects while, GML19 showed the highest positive and non-significant (p>0.05) SCA effect when crossed to PA-1. On the contrary, GML 57 followed by GML26, GML47 and GML13 had the highest significant (p<0.05) and negative SCA effect.

The SCA effects for the lines crossed with P1 are shown in Figure 4.3. Nineteen lines demonstrated a positive SCA effect and 21 lines had a negative SCA effect when crossed to P1. GML57 followed by GML26, GML47 and GML28 displayed the highest positive and significant (p<0.05) SCA effects. Conversely, GML18 followed by GML3 showed the highest negative and significant (p<0.01) SCA effects and GML19 and GML1 exhibited the highest negative and non-significant SCA effects.

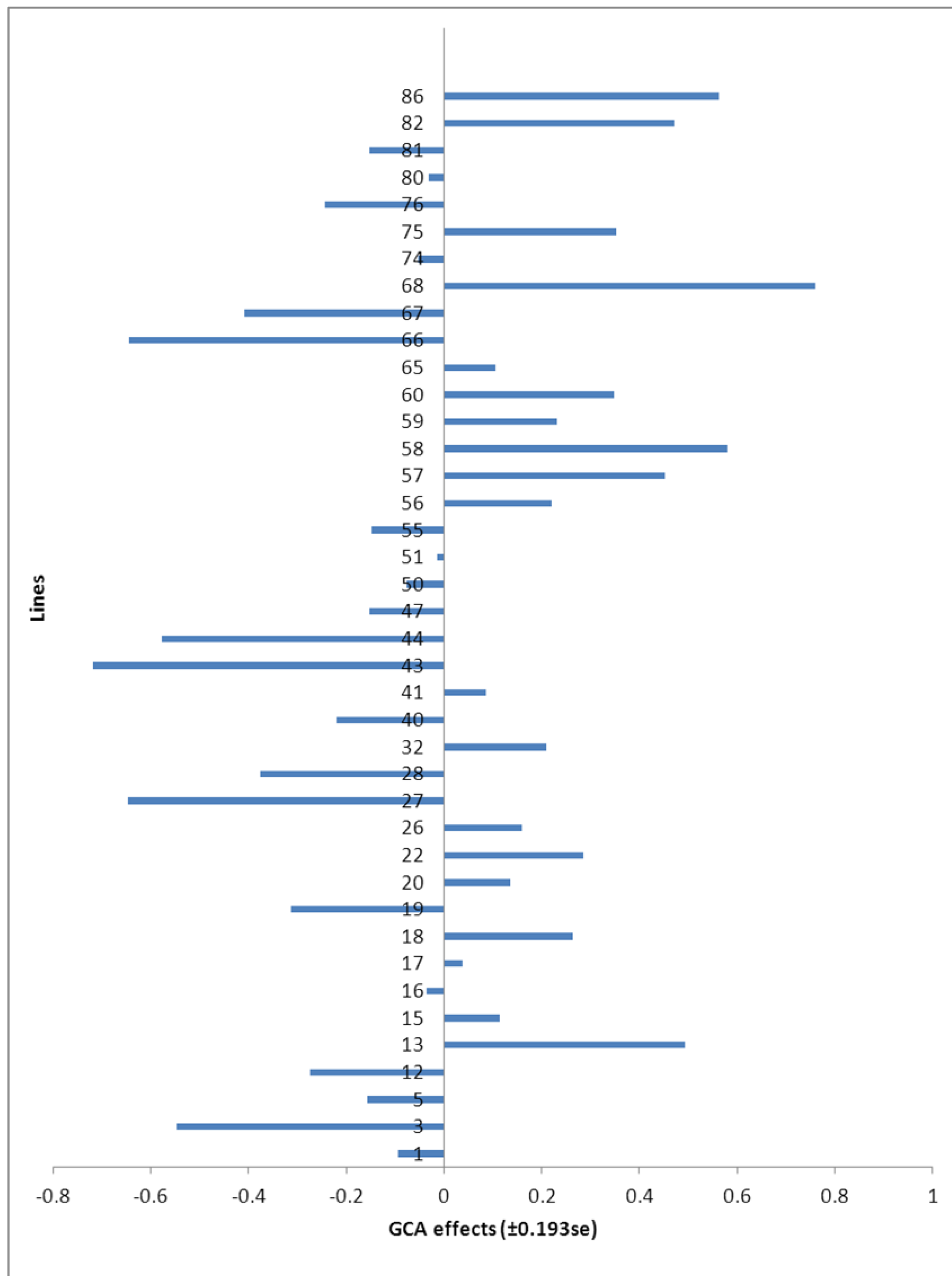

Figure 4.1: General combining ability effects of 40 maize inbred lines

(se= standard error)

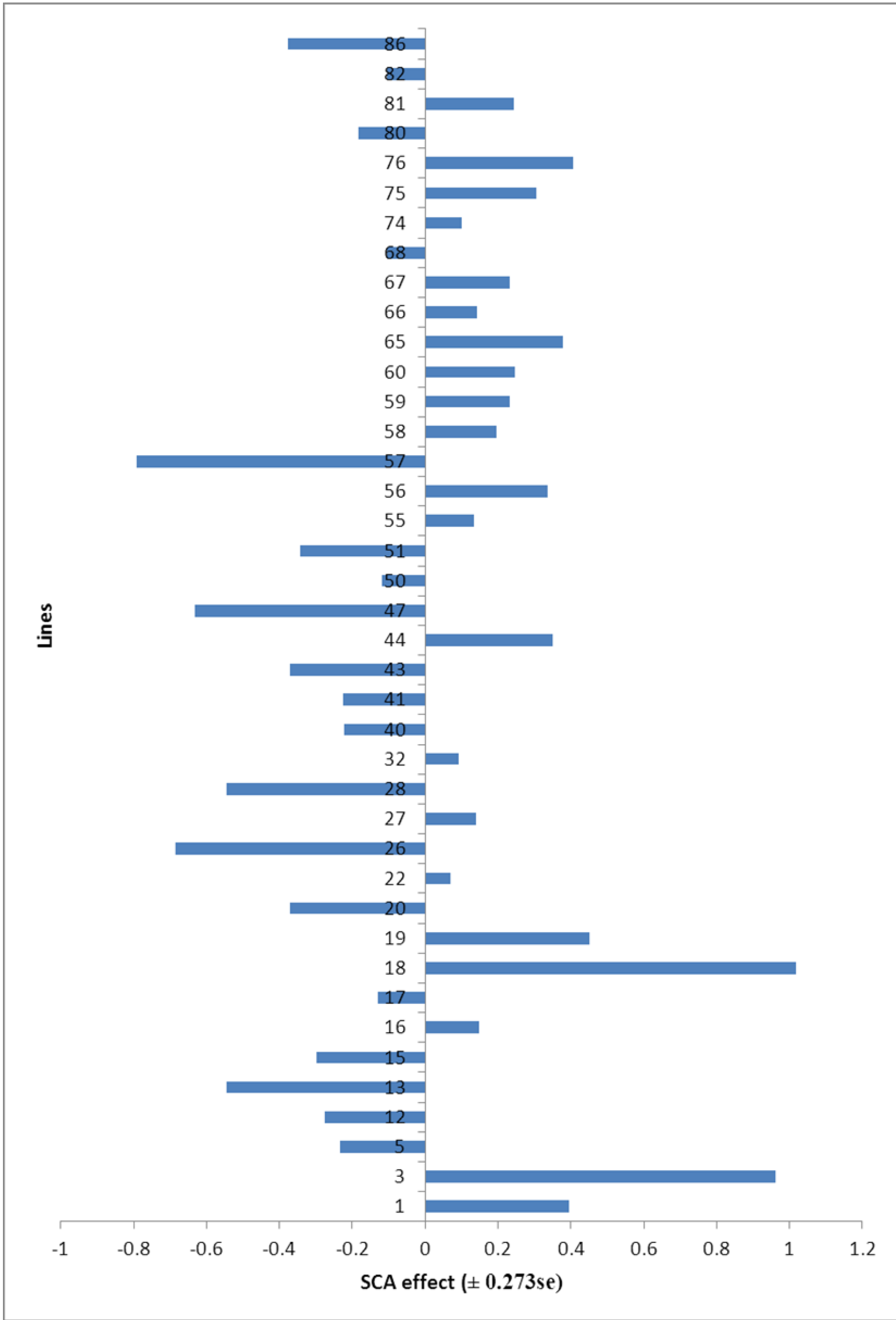

Figure 4.2: Specific combining ability effects of 40 maize inbred lines crossed to tester PA-1

(se= standard error)

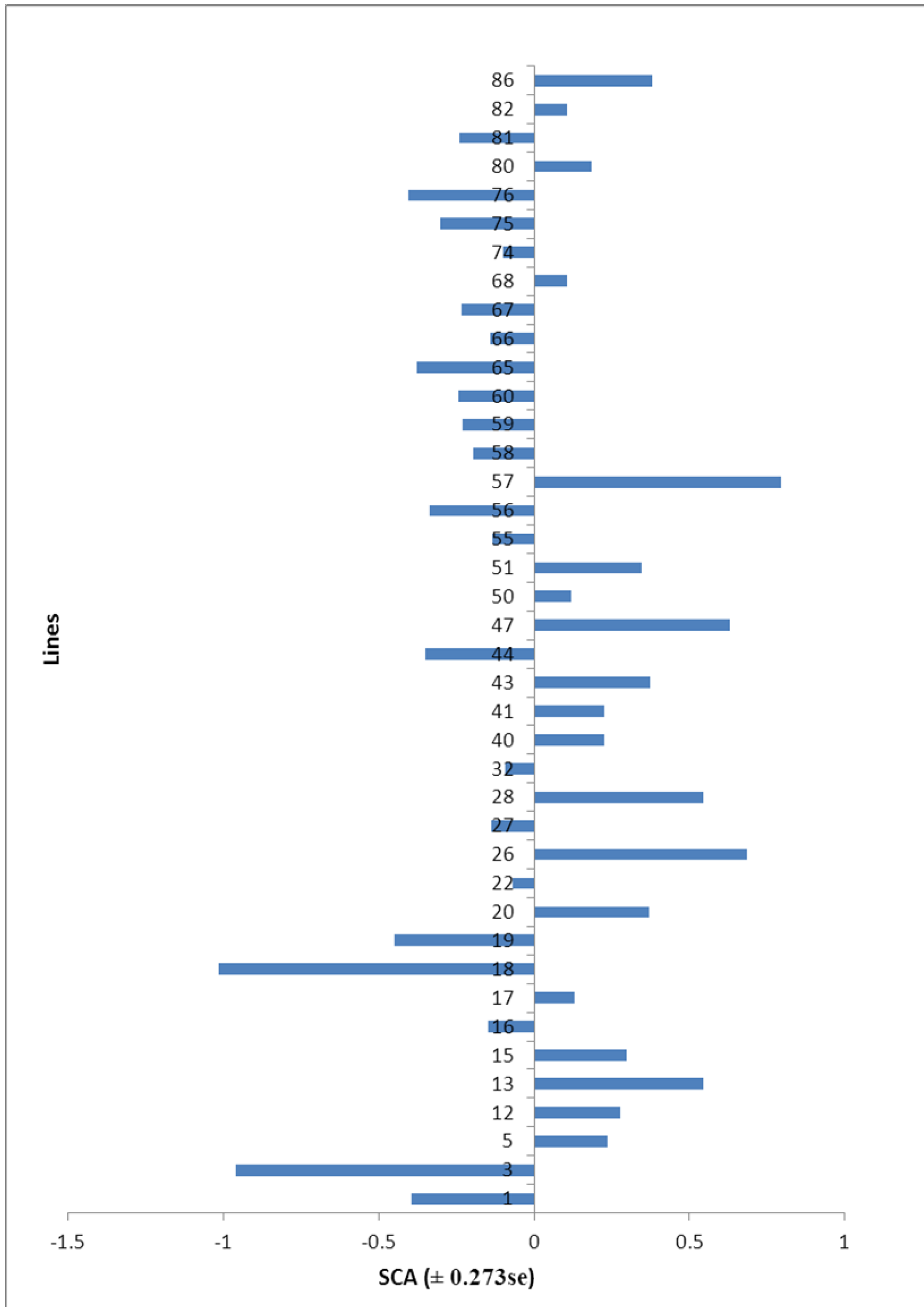

Figure 4.3: Specific combining ability effects of 40 maize inbred lines crossed to tester P1 (se= standard error)

4.3.3 Genetic parameters for yield and associated traits

The data for genetic parameters is based on all hybrids that were evaluated in trials. The heritability of grain yield was the highest at 10Dundee and lowest at 10Cedara. Heritability across environment was 84.16% and it ranged from 85.69% to 94.07% between environments. The heritability of number of ears per plant was the highest at 12Ukulinga and lowest at 10Makhathini. Genetic advance ranged from 1.77 to 3.59 for grain yield, however genetic advance as a percentage of mean ranged from 51.36 to 119.27 across environments. For number of ears per plant, genetic advance ranged from 0.00 to 0.19, but genetic advance as a percentage of mean ranged from 0.00 to 26.12 across environments (Table 4.2).

Table 4.2: Genetic parameters of grain yield and number of ears per plant

Genetic parameters	Across environment	10Cedara	10Dundee	10Makhathini	12Dundee	12Ukulinga
			Grain yield (t ha^{-1})			
σ^2G	0.67	0.68	1.86	0.86	0.89	0.88
σ^2E	1.25	1.14	1.17	0.69	1.08	0.88
σ^2p	1.92	1.82	3.03	1.55	1.97	1.76
H^2 (%)	84.16	85.69	94.07	92.51	89.20	90.9
σ_p	2.12	1.60	1.74	1.29	1.40	0.95
GA	3.59	2.83	3.38	2.47	2.57	1.77
Mean (t ha^{-1})	3.64	5.51	5.39	2.88	2.85	1.49
GAM (%)	98.68	51.36	62.76	85.51	90.56	119.27
GCV (%)	22.41	15.01	25.35	32.1	33.23	62.98
PCV (%)	38.07	24.48	32.32	43.12	49.33	89.04
			Number of ears per plant			
σ^2G	*	0.01	*	0.00	0.02	0.01
σ^2E	*	0.04	*	0.07	0.73	0.04
σ^2p	*	0.05	*	0.07	0.75	0.05
H^2 (%)	*	34.7	*	0.00	4.29	38.19
σ_p	*	0.27	*	0.27	0.89	0.24
GA	*	0.19	*	0.00	0.08	0.18
Mean	*	1.21	*	0.72	1.52	0.70
GAM (%)	*	15.78	*	0.00	5.08	26.12
GCV (%)	*	8.81	*	0.00	40.67	25.84
PCV (%)	*	18.47	*	36.75	56.97	31.94

*Data not recorded, 0.00 data was negative, σ^2G= Genotypic variance, σ^2E= Environmental variance, σ^2p= Phenotypic variance, H^2=Broad sense heritability, σ_p= standard deviation, GA= Genetic advance, GAM = Genetic advance as a percentage of mean, GCV=Genotypic Coefficient of variation and PCV= Phenotypic Coefficient of variation Values.

The heritability of grain moisture was highest at 12Ukulinga and lowest at 10Makhathini. In addition, heritability ranged from 0.00% to 67.28% between environments (Table 4.3). The heritability of anthesis date was highest at 10Dundee and lowest at 10Makhathini. The heritability of plant height was highest at 12Ukulinga and lowest at 10Makhathini. Genetic advance ranged from 0.00 to 1.02 for grain moisture, however genetic advance as a percentage of mean ranged from 0.00 to 7.20 across environments. Additionally, genetic advance ranged from 0.00 to 2.66 for anthesis date and genetic advance as a percentage of mean ranged from 0.00 to 3.57 across environments. For plant height, genetic advance ranged from 0.39 to 17.93, nevertheless genetic advance as a percentage of mean ranged from 0.17 to 7.88 across environments. Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all traits in all environments.

Table 4.3: Genetic parameters of secondary traits across and within environments

Genetic parameters	Across environment	10Cedara	10Dundee	10Makhathini	12Dundee	12Ukulinga
			Grain moisture (%)			
σ^2G	*	0.81	0.13	0.00	*	0.23
σ^2E	*	8.76	2.19	21.73	*	0.22
σ^2p	*	9.57	2.32	21.73	*	0.45
H^2 (%)	*	15.59	10.26	0.00	*	67.28
σ_p	*	3.18	1.51	4.35	*	0.67
GA	*	1.02	0.32	0.00	*	0.90
Mean (%)	*	21.24	20.19	15.86	*	12.57
GAM (%)	*	4.80	1.58	0.00	*	7.20
GCV (%)	*	4.23	1.75	0.00	*	4.63
PCV (%)	*	14.56	7.54	29.39	*	5.34
			Anthesis date (days)			
σ^2G	*	0.81	0.72	0.00	*	2.13
σ^2E	*	2.88	1.11	5.21	*	4.50
σ^2p	*	3.69	1.83	5.21	*	6.63
H^2 (%)	*	35.98	56.21	0.00	*	48.7
σ_p	*	2.40	1.35	2.18	*	2.72
GA	*	1.78	1.56	0.00	*	2.66
Mean (days)	*	66.74	83.57	61.27	*	74.59
GAM (%)		2.66	1.86	0.00	*	3.57
GCV (%)	*	1.35	1.01	0.00	*	2.81

PCV (%)	*	2.88	1.62	3.72	*	3.45
Plant height (cm)						
σ^2G	*	78.60	*	1.70	39.80	82.10
σ^2E	*	374.80	*	387.80	441.10	222.30
σ^2p	*	453.40	*	389.50	480.90	304.40
H^2 (%)	*	29.55	*	0.87	15.29	42.48
σ_p	*	23.48	*	21.58	22.45	21.00
GA	*	14.29	*	0.39	6.90	17.93
Mean (cm)	*	286.73	*	226.43	209.3	227.71
GAM (%)	*	4.98	*	0.17	3.30	7.88
GCV (%)	*	3.09	*	0.58	7.71	6.10
PCV (%)	*	7.43	*	8.72	10.47	7.66

*****Data not recorded, 0.00 data was negative, σ^2G= Genotypic variance, σ^2E= Environmental variance, σ^2p= Phenotypic variance, H^2=Broad sense heritability, σ_p= standard deviation, GA= Genetic advance, GAM= Genetic advance as a percentage of mean, GCV=Genotypic Coefficient of variation and PCV= Phenotypic Coefficient of variation Values

4.3.4 The frequency distribution of hybrids

Two different environments from each of the 2 major clusters (refer to Chapter3) were selected to observe the distribution of traits, under favourable and unfavourable conditions, of 10Cedara and 12Ukulinga respectively.

Grain yield showed a continuous distribution in both environments. Grain yield was normally distributed at 10Cedara, however, grain yield at 12Ukulinga was skewed to the right meaning that most of the genotypes were low yielding, 1 to 1.5 t ha^{-1} (Figure 4.4). In addition, many genotypes at 10Cedara yielded between 4 and 7 t ha^{-1}. Furthermore, anthesis date was normally distributed at 12Ukulinga and skewed to the right at 12Cedara, meaning that many genotypes were early (65-68 days) at 10Cedara and late (73-75 days) at 12Ukulinga. However, the distribution of hybrids was continuous at 12Ukulinga and non-continuous at 10Cedara (Figure 4.5).

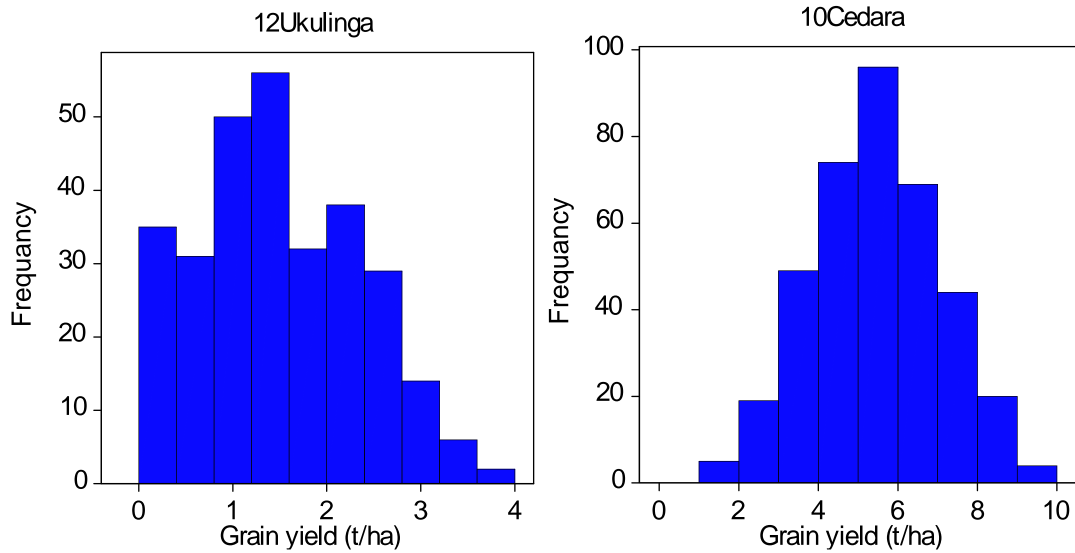

Figure 4.4: The histogram of grain yield for maize hybrids across two environments

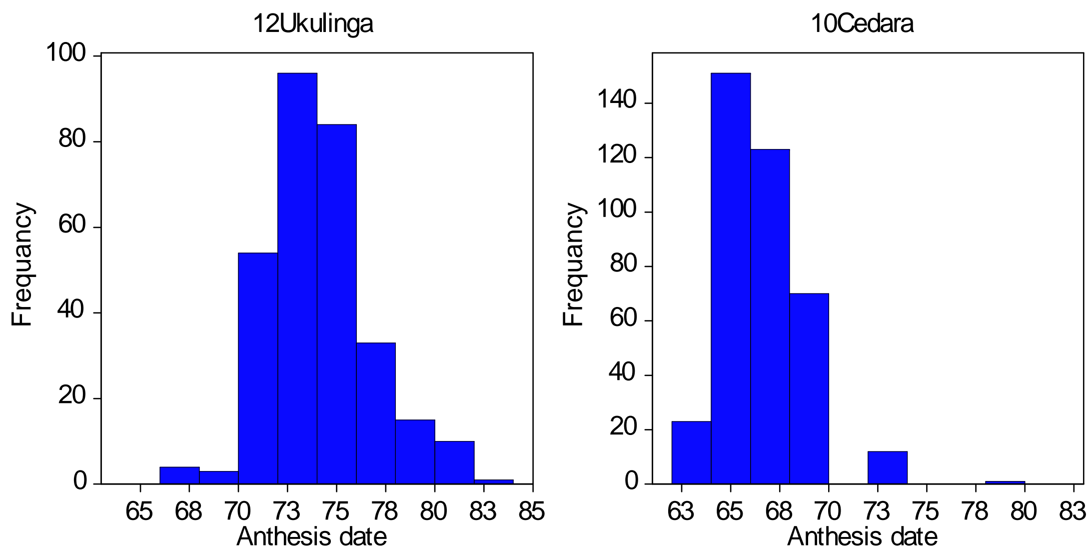

Figure 4.5: The histogram of anthesis date for maize hybrids across two environments

Numbers of ears per plant was normally and continuously distributed at 12Ukulinga and 10Cedara; however the number of genotypes with more than two ears was highest (1 to 1.5) at 10Cedara and lowest (0.5-1.0) at 12Ukulinga (Figure 4.6).

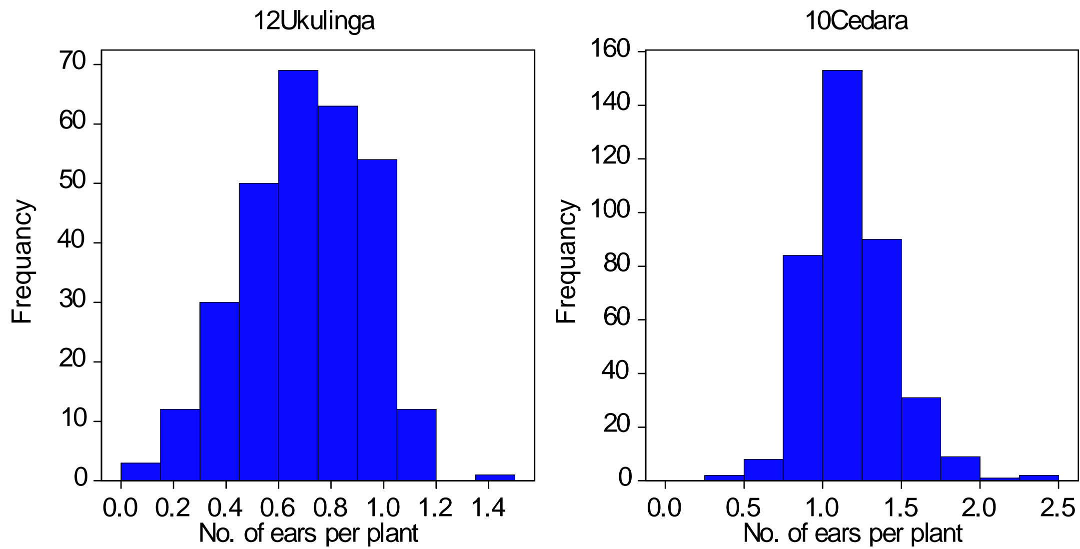

Figure 4.6: The histogram of number of ears per plant for maize hybrids

Plant height showed a continuous distribution in both environments, many genotypes were very tall (250-325cm) at 10Cedara and many were short (220-240cm) at 12Ukulinga (Figure 4.7).

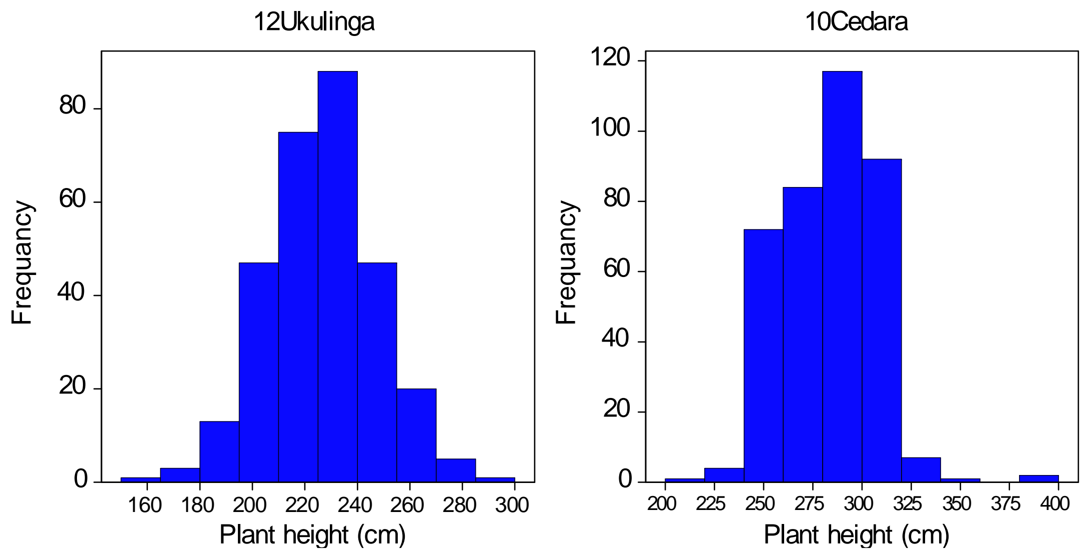

Figure 4.7: The histogram of plant height for maize hybrids across two environments

4.3.5 The relationship between yield and secondary traits in maize hybrids

Two different environments from each of the 2 clusters representing favourable and unfavourable conditions, of 10Cedara and 12Ukulinga, respectively (refer to Chapter 3) were selected to perform phenotypic correlations between traits.

Grain yield exhibited highly significant (P<0.01) and positive association with number of ears per plant and plant height in both environments. There was a significant (P<0.01) positive association between grain yield and grain moisture at 12Ukulinga. However, the correlation between grain yield and grain moisture was negative and non-significant at 10Cedara. In contrast, grain yield showed a highly significant (p<0.01) and negative association with anthesis date, anthesis-silking interval and silking date at 12Ukulinga and negative (p<0.01) correlation between grain yield and anthesis date and anthesis-silking interval (p>0.05) at 10Cedara. Grain yield was positive and significantly correlated with stem lodging at 12Ukulinga, however, the opposite was observed at 10Cedara. Grain yield exhibited highly significant (P<0.01) positive association with number of plants at 12Ukulinga, nevertheless, the correlation was positive but not significant at 10Cedara. Grain yield showed a highly significant (p<0.01) and negative association with root lodging in both environments, however, at 12Ukulinga the correlation was insignificant. There was a positive and significant (p<0.01) association between grain yield and grain texture at 10Cedara (Table 4.4 and Table 4.5).

Table 4.4: Relationship between yield and secondary traits under low input conditions (12Ukulinga)

Traits	Anthesis silking interval (days) (ASI)	Anthesis date (days) (AD)	Silking date (days) (SI)	Ear height (cm) (EH)	Ear position (EPO)	No. of ears per plant (EPP)	Grain moisture (%) (GM)	No. of plants (NP)	Plant height (cm) (PH)	Root lodging (%) (RL)	Stem lodging (%) (SL)	Grain yield (t ha^{-1}) (GYLD)
ASI	-											
AD	0.3569**	-										
SI	0.8515**	0.7937	-									
EH	-0.2234**	-0.0455	-0.171**	-								
EPO	-0.0652	0.0747	-0.0006	0.6834**	-							
EPP	-0.2239**	-0.0384	-0.1673**	-0.0253	-0.11	-						
GM	-0.3061**	-0.0147	-0.2076**	0.189**	0.098	0.3073**	-					
NP	-0.0631	-0.2453**	-0.1788**	0.0757	0.0411	0.0141	0.0136	-				
PH	-0.2462**	-0.1233*	-0.2296**	0.7697**	0.063	0.0589	0.1686*	0.0606	-			
RL	0.0839	0.163**	0.1461*	-0.2266**	-0.1907**	0.037	-0.0839	-0.1565**	-0.1385*	-		
SL	-0.3174**	-0.3209**	-0.3868**	0.2674**	0.2359**	0.0746	0.2029**	0.0774	0.1546**	-0.304**	-	
GYLD	-0.3979**	-0.3083**	-0.4322**	0.0566	-0.1555	0.7176**	0.4079**	0.2863**	0.2138**	-0.0118	0.246**	-

*Data significant at $p<0.05$; **Data highly significant at $p<0.01$

Table 4.5: Relationship between yield and secondary traits under high input conditions (10Cedara)

Traits	Anthesis date (days) (AD)	Anthesis-silking interval (ASI)	Ear height (cm) (EH)	Ear position (EPO)	No. of ears per plant (EPP)	Ear length (cm) (EL)	Grain moisture (%) (MOI)	Plant height (cm) PH	Root lodging (%) (RL)	Stem lodging (%) (SL)	Grain texture (%) TEX	Grain yield (t ha^{-1}) (GY)
AD	-											
ASI	-0.0917	-										
EH	0.076	-0.0049	-									
EPO	0.1884**	0.0124	0.7225**	-								
EPP	-0.1868**	-0.1263*	0.1778**	0.0253	-							
EL	-0.1882**	0.0439	-0.019	-0.2416**	0.0727	-						
MOI	0.215**	0.0178	-0.0493	0.009	-0.2167**	0.1226	-					
PH	-0.131*	-0.0138	0.4218**	-0.3153**	0.2011**	0.3002**	-0.0763	-				
RL	0.1545**	-0.0197	0.031	0.0661	-0.0431	-0.1489**	0.0216	-0.0373	-			
SL	0.0208	-0.1631**	-0.054	0.0131	0.1278	-0.1556	-0.1475*	-0.0955	0.1001	-		
TEX	-0.2042**	-0.0091	0.0389	-0.0433	0.1172	0.1017*	-0.0146	0.1157*	-0.1066*	-0.0994	-	
GY	-0.3668**	-0.0643	0.0557	-0.2318**	0.4768**	0.4981**	-0.0942	0.3721**	-0.173**	-0.1508**	0.2577**	-

*Data significant at $p<0.05$; **Data highly significant at $p<0.01$

4.4 Discussion

4.4.1 Combining ability effects

The line X tester analysis revealed the type of gene action that contributed to the inheritance of grain yield. The significant difference of the lines and testers ($p<0.01$) was an indication that the lines behaved differently in their respective crosses and that greater diversity existed between both the testers and lines. Moterle et al. (2011) reported that genetic diversity is extremely important for obtaining genetic gain through creating hybrids. In the current study, Line X tester interaction was highly significant ($p<0.01$), this indicated that different lines may have different combining patterns depending on the tester used. The interaction between the line X environment and tester X environment was also highly significant ($p<0.01$), this suggests that inbred lines and testers performed differently in their respective environments. The interaction between the line X testers X environment was also significant ($p<0.05$) confirming the possibly complications caused by GXE as reported in chapter 3. This observation of significant GXE is consistent with previous findings reported by Packer (2007), Shashidhara (2008), Fan et al. (2010) and Aly et al. (2011). Significance of lines and tester's main effects, and line X tester interaction, indicates that GCA due to lines, GCA due to the testers and SCA effects, respectively, were important in explaining difference between hybrids. This also suggests that both additive and non-additive gene action were important in governing grain yield. However, additive gene action due to testers contributed more to the inheritance of grain yield, than the lines. This can be explained by the fact that the lines were derived from a bi-parental population with a narrow genetic base, whereas the testers were quite divergent.

General combining ability effects

Highly significant ($p<0.01$) positive GCA effects were found in GML58 and GML68 and significant ($p<0.05$) positive GCA effects were observed for GML13 and GML86, so these lines can be regarded as good general combiners for grain yield and hence are desired. Conversely, highly significant negative GCA effects were recorded for GML27, GML43, GML44 and GML66 and can thus be considered poor general combiners (non-desired) and cannot be recommended for advancement. The GCA effects for tester P1 was positive and

significant (p<0.05) suggesting that it is a good parent with a desired GCA effects, this is mainly because this tester is tolerant to drought and diseases. In contrast, tester PA-1 exhibited significant (p<0.05) and negative GCA effects indicating that it is a poor parent with an undesired GCA effects, this is because it is very susceptible to stress like drought and only manage to perform well under favourable conditions.

Specific combining ability effects

In this study, 19 lines had positive SCA effects for grain yield when crossed to tester P1 and 21 lines exhibited a positive SCA effects when crossed to tester PA-1, suggesting that there is potential for developing high yielding hybrids. Our study revealed highly significant (p<0.01) positive SCA effects when GML26 and GMH57 (high positive GCA effect) were crossed to P1 (high positive GCA effect), this was highly expected and it confirmed that additive gene action played a significant role in conferring grain yield. Furthermore, highly significant (p<0.01) positive SCA effects were found, when GML18 (positive GCA effect) was crossed to tester PA-1 (negative GCA effect), this imply that they had a good specific combining ability, in other words they complement each other very well. This could also mean that the line and tester belong to different heterotic groups, hence possess different gene frequency for grain yield. This is in accord with Uddin et al. (2006) who found that high and low combiners can give crosses with positive SCA effects. Furthermore, in the current study, highly significant (p<0.01) positive SCA effects were found when GML3 (negative GCA effect) was crossed to PA-1 (negative GCA effect); hence these lines have good specific combining ability and thus are desired; this was not expected as both the line and the tester had negative GCA effects. Therefore, it could be concluded that in addition to the additive effects the dominance gene effects played a significant role in this study, meaning that yield was enhanced by interaction between GML3 and tester PA-1 in the hybrids. This is confirmed from the overall analysis (Table 4.1) because Lines, testers and Line X tester interaction contributed 15.6%, 65.4% and 19.1%, respectively, to the inheritance of grain yield. Amiruzzaman et al. (2011) pointed out that generally GCA effects of the parents did not reflect in their SCA effect for all the traits studied. Furthermore, Amiruzzaman et al. (2011) reported that the SCA effects is a result of the interaction of GCA effects of the parents and that it can improve or deteriorate the hybrid expression compared to the

expected effect based on GCA effects only. Therefore, the results of the current study suggest that the hybrids with positive SCA effects could be utilized in heterosis breeding to exploit hybrid vigour.

Additionally, when GML18 (good GCA effects) was crossed to the good tester (P1) it showed a significant negative SCA effect, this was not expected, and thus it indicated that the genes of the two good parents did not contribute to yield by adding up their good effects. Uddin et al. (2006) found similar results and came to a conclusion that good general combining parents do not always show high SCA effects in their hybrid combinations. These results are in agreement with Amiruzzaman et al. (2011) who reported that all the studied traits were mainly governed by both additive and non-additive gene action. However, Shashidhara (2008) reported that grain yield was predominantly governed by non-additive gene action. In contrast, Aly et al. (2011) reported that additive gene action played an important role than non-additive gene action in the inheritance of grain yield which is consistent with findings in the current study. To elucidate, Ojo et al. (2007) reported that this discrepancy can be explained by the difference in genetic material used in the study. It could be caused by the different genetic background of the tested genetic material, the effect of inbreeding, as well as the environmental factors.

4.4.2 The frequency distribution of hybrids for yield and secondary traits

The results revealed that the distribution of grain yield and secondary traits varied from one environment to another due to significant GXE effects. Many genotypes at 10Cedara were high yielding, whereas, many were low yielding at 12Ukulinga. This is because 10Cedara was a favourable environment; in contrast to 12Ukulinga which was unfavourable due to drought occurring at the sensitive flowering stage (refer to chapter 3, Figure 3.1 and 3.2). Many genotypes produced pollen early at 10Cedara (65-68 days) and many genotypes produced pollen late (73-75 days) this is again due to drought occurring during the flowering stage at 12Ukulinga. Further, many genotypes at 10Cedara were prolific whereas many genotypes at 12Ukulinga were not prolific, this indicates that prolificacy on these genotypes is unstable; its expression depends on the conditions of the environments. At 10Cedara many plants were taller than genotypes at 12Ukulinga; this is not desired as taller genotypes are more prone to lodging than short ones. All the histograms show continuous distribution

of hybrids for these quantitative traits, which confirms involvement of many genes in their control.

4.4.3 Genetic parameters of grain yield and secondary traits

The heritability percentage was categorized as low, moderate and high in accordance with Robinson et al. (1949) as follows: 0-30%: Low, 30-60%: Moderate, and >60%: High.

I. Grain yield

The heritability of grain yield was generally higher in this study as it ranged from 85.69% and 94.07% between environments. The results indicate that grain yield was not highly affected by the environment and has a high response to selection. This has implications that direct selection can be recommended over indirect selection. These results are in accordance with Mahmood et al. (2004) who also reported highest heritability of 99.3% for grain yield and Nadagoud (2008) who reported heritability of 96.80%. Though, Ali et al. (2011) found a high heritability for grain of 67%. These results are in contrast with Asghar and Mehdi (2010) who reported a heritability of 38%. Although grain yield had the highest heritability its genetic advance was low, this implies that there is still a need to improve the efficiency of selection for yield. Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the environments; this indicates the significant genotype x environment interactions for grain yield (Table 4.2) which is in consisted with findings by Nadagoud (2008).

II. Secondary traits

The heritability of number of ears per plant, grain moisture and plant height was generally low in this study, the highest at 12Ukulinga (39.19 %, 67.28% and 42.48%) respectively and lowest at 10Makhathini (0.00%, 0.00% and 0.87%) respectively. This again implies that these traits are suitable secondary trait to improve grain yield only under unfavourable conditions. Similarly, Magorokosho et al. (2003) and Ali et al. (2011) reported heritability of 42% for number of ears per plant in a drought stressed environment. Furthermore, Ali et al. (2011) found a moderate heritability of 37% for grain moisture in testcrosses at harvest. The heritability of anthesis date was the highest at 10Dundee (56.21%) and lowest at

10Makhathini (0.00%), this shows that this trait is a suitable secondary trait to improve grain yield under favourable conditions. There was no genetic variation for grain yield in 10Makhathini because the hybrids flowered almost at the same time. Similarly, Kabdal et al. (2003) reported low heritability of 39.23% for anthesis date in a diallel cross of maize. On the contrary, Mahmood et al. (2004) and Nadagoud (2008) reported a high heritability of 91.3% and 89.27% respectively for anthesis date. Plant height had the largest genetic advance and number of ears per plant had the lowest genetic advance. This shows that there was high genetic improvement for plant height. In addition, there is a great need to improve the performance of the other traits. The higher phenotypic coefficient of variation over genotypic coefficient of variation for all this traits indicated the significant genotype x environment interactions in the expression of these traits. Similar results were observed by Manigopa and Rameswar (2012) who found that PCV was higher than GCV for many traits.

4.4.4 The relationship between yield and secondary traits in maize hybrids

Grain yield exhibited highly significant ($P<0.01$) and positive association with plant height, ear length, grain moisture and grain texture. This implies that increasing expression of these traits can positively influence grain yield. Moreover, positive relationship indicates that the favourable genes controlling these traits present in the parents could be utilized for improvement of these lines in future breeding programs. In addition, significant ($p<0.01$) correlation was found between plant height and grain yield in both environments. Also, grain yield exhibited negative association with ear position in both environments though not significant in 12Ukulinga. This indicates that these traits can be effective for indirect selection of grain yield as their association with yield is not greatly influenced by the environment. The results are in agreement with Selvaraj and Nagarajan (2011) who found that plant height, ear height and ear length showed significant positive association with yield. This is similar to findings by Betrán and Hallauer (1996), Kabdal et al. (2003), Monneveux et al. (2008) and Ilker (2011).

Grain yield was strongly correlated with number of ears per plant in both environments but the correlation was stronger in 12Ukulinga (0.77) than 10Cedara (0.47). This is expected as the more ears a plant can produce the higher yielding it is likely to be. Additionally, Munyiri et al. (2010) reported that number of ears per plant is one of the most important yield

components of maize. This also indicates that under stressful conditions (12Ukulinga) prolificacy contributed more to yield. The results are in accord with Monneveux et al. (2008) who reported that under drought conditions, grain yield was strongly positively correlated with number of ears per plant. In addition, Magorokosho et al. (2003) reported that the relationship between number of ears per plant and grain yield became stronger with increasing moisture stress. This also shows that this trait is not greatly influenced by the environment and can thus be used as a secondary trait for indirect selection of yield, since only the magnitude changed and the direction remained unchanged.

In contrast, grain yield showed a highly significant ($p<0.01$) negative association with anthesis date, anthesis-silking interval and silking date in 12Ukulinga. Similarly, grain yield and anthesis date were significant and negatively correlated in 10Cedara. These results showed that earliness rather than lateness had the potential of increasing grain yield. This also implies that this trait is less influenced by the environment, it is however, more influenced by genetic factors and thus it is more heritable which makes it a suitable trait to use when selecting for grain yield indirectly. This is in contrast with Selvaraj and Nagarajan (2011) who reported that anthesis date and silking date showed positive non-significant association with grain yield. However, the current study results were in agreement with Muhammad et al. (2011) who reported a negative association between anthesis date and silking date with grain yield both at genotypic and phenotypic levels. In addition, Kumar et al. (2011) reported a highly significant negative correlation between anthesis date and silking date with yield per plant. There was a strong correlation between anthesis date and silking date in 12Ukulinga. This means that selecting for anthesis date will change silking date by the same magnitude. This is in agreement with Olakojo and Olaoye (2011) who reported a correlation of 0.74 between anthesis date and silking date.

Grain yield showed a highly significant ($p<0.01$) and negative association with root lodging in 10Cedara, however, in 12Ukulinga the correlation was negative and non-significant. This is because if the plant is root lodged its ability to extract nutrients from the soil is reduced. There was a negative and high significant ($P<0.01$) correlation between stem lodging and root lodging in 12Ukulinga, however, the correlation was positive and non-significant ($P>0.05$) in 10Cedara. This is because root lodging is mainly associated with environmental

factors such as heavy rains coinciding with wind or management factors such as high densities or poor plant distribution, whereas stalk lodging is related to genetic characteristics such as disease and insect resistance, prolificacy and senescence patterns (Mariano et al., 2007).

4.5 Conclusion

From the study it can be concluded that,

- P1 and PA-1 were able to discriminate the new inbred lines according to GCA and SCA for yield implying that they can be used to develop hybrids in future breeding. This provided the opportunity to select the best inbreds.
- GML18 X tester PA-1 and GML3 X tester PA-1 exhibited good specific combining ability; this suggests that these hybrids could be utilized in heterosis breeding to exploit hybrid vigour.
- Both additive and non-additive gene action contributed significantly to the inheritance of grain yield, however, additive gene action due to testers had the major contribution, which confirms the differences between the testers. Inbred lines showed small differences because they were derived from a common bi-parental population with a narrow genetic base.
- For indirect selection strategy, number of ears per plant, anthesis date and plant height are the secondary traits which are suitable to be used in the improvement of grain yield, because their correlation with grain yield had the same direction in both stressed and non-stressed environment. Additionally, their heritability was moderate. However, direct selection for grain yield would be recommended due to its high heritability in all environments compared to that of secondary traits.

The results obtained would be useful in devising the most appropriate breeding strategy to enhance yield of hybrids.

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CHAPTER 5

OVERVIEW OF THE RESEARCH FINDINGS

5.1 Introduction

This chapter makes an overview of the completed study by summarizing the major objectives and highlighting the major findings. The implications of these findings and recommendations are discussed.

The specific objectives of the study were as follows:

a) To investigate genetic diversity using SSR molecular markers and phenotypic data in a set of 60 maize inbred lines from the program at UKZN
b) To determine genotype by environment interaction in white maize hybrids
c) To determine cultivar superiority of new maize hybrids
d) To determine combining ability of new maize inbred lines
e) To determine the relationship between secondary traits with yield in white maize hybrids
f) To determine genetic parameters such as heritability, genotypic and phenotypic coefficient of variation in the maize hybrids

5.2 Major findings

The major findings on the levels of diversity, combining ability and cultivar superiority are presented below.

5.2.1 Genetic Diversity

There is significant diversity between both the experimental lines and the testers:

- There was a greater variation between testers and lines, the genetic distances ranged from 0.19 to 0.83 between the new lines, and 0.29 to 0.83 between the lines and testers.

- On the basis of cluster analysis using SSR markers, these 60 lines were classified in two major groups and then further divided into seven sub groups.
- The results showed that inbred lines which were put in the same cluster were related by pedigree and origin, which is consistent with previous findings.
- Furthermore, cluster analysis based on phenotypic data classified inbred lines into two major clusters and four sub-clusters, it showed that inbred lines in the same group as well as in the sub-groups were similar in their physical and phenotypic characters.
- The clustering of genotypes using morphological data was similar to the clustering of genotypes using SSR markers for other inbred lines and different in others. The discrepancy of these results was mainly due to genotype x environment interaction which can mask genetic differences in the phenotypic data.
- Hence, the SSR markers were more accurate in clustering inbred lines since they are not influenced by the environment.
- Inbred lines which were clustered on the same group could be allocated to the same heterotic group and inbred lines on different clusters could be allocated in different heterotic groups.
- Hence, seven heterotic groups were identified based on SSR markers. It is concluded that high genetic diversity exist in the germplasm which implies the potential of producing superior genotypes.

5.2.2 Cultivar Superiority

The study identifies superior hybrids which should be advanced in the breeding programme:

- The results revealed significant differences between hybrids and environments as main effects and their interaction. In this study, only the IPCA1 and IPCA2 were significant, hence the AMMI-2 model was adopted.
- The hybrid GMH113 was the most adaptable genotype in all environments, but specifically adaptable to high yielding environments. GMH2 was specifically adapted to high yielding environments.
- Five superior genotypes were identified (GMH146, GMH113, GMH170, GMH155 and GMH124). It was concluded that AMMI-2 is a good model to use to select for

superior genotypes and best environments for genotype evaluation as this was confirmed by clustering of genotypes in a dendrogram.

- Observation of significant G X E, especially the cross-over type indicates that genotypes need to be tested in several years and locations to identify superior and stable hybrids.

5.2.3 Combining Ability and Genetic Variation

The completed study also confirms existence of significant variation for combining ability which can be exploited in the breeding programme:

- Results indicate significant line and tester main effects and line X tester interaction effects ($p<0.01$), implying that both GCA which is attributable to both lines and testers, and SCA effects, respectively, are important in governing grain yield.
- Four lines GML68, GML58, GML86 and GML13 displayed large and significant GCA effects for yield. Five crosses with large positive SCA effects for yield were identified.
- It was also observed that crosses involving lines with negative GCA also gave hybrids with positive SCA effects indicating that dominance gene action played a significant part in influencing the yield of hybrids.
- Additive gene action contributed more to the inheritance of grain yield in the hybrids than the non-additive gene portion.
- Heritability of grain yield was the highest in all environments ranging from 85 to 94%, whereas heritability for the secondary traits such as number of ears per plant, anthesis date, grain moisture and plant height ranged from low to high.
- Consistent with the literature there was a significant relationship between grain yield and most of the secondary traits, with implication for breeding strategy.
- It can be concluded that the number of ears per plant and anthesis date could be the most reliable secondary traits to improve yield via indirect selection, because they were highly heritable in both low and high yielding environments.
- However, the observation of large heritability estimates for grain yield in this set of germplasm lines supports direct selection strategy for enhancing grain yield.

5.3 Closing remarks: Implications for breeding and the way forward

Food security challenges in Sub-Saharan Africa (SSA) which are presented by climate changes that are reflected by global warming, increasing frequency of drought in maize production areas in South Africa calls for development of new maize systems that can cope with climate change. This can be achieved through integration of both conventional and molecular breeding. Although, molecular marker selection has been proved to be reliable, quick and not influenced by the environment, its success also depends on conventional plant breeding. The biggest challenge is to bridge the gap between molecular breeding and conventional plant breeding. The results from the concluded study revealed high genetic diversity in the inbred lines, implying that there is a high potential for producing new and superior hybrids. However, low genetic base was observed among the lines used in the combining ability study, this was expected as modern plant breeding results in homogeneity of varieties due to restrictive germplasm used. This suggests that it is very necessary to introduce exotic germplasm into local germplasm for enrichment of the genetic base. The establishment of heterotic patterns has implication that appropriate parental lines for hybrid combinations can be selected. There was a highly significant genotype X environment interaction, particularly the crossing over interaction which was observed, indicating the need to evaluate genotypes in different locations and years. The study divided the environments into two groups, high and low yielding environments which provide the opportunity to select the hybrid within the two groups for recommendation to favorable and unfavorable environments which are represented by these test environments, respectively. The identified potential heterotic groups, the predominance of both additive and non-additive effects, for grain yield in new maize germplasm lines, can be exploited to develop new varieties. It is recommended that heterosis can be studied in the inbred lines form the same and different heterotic groups.